Biological counterstrike: antibiotic resistance mechanisms of Gram-positive cocci

N. Woodford

Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency, London NW9 5HT, UK

ABSTRACT

The development of antibiotic resistance by bacteria is an evolutionary inevitability, a convincing demonstration of their ability to adapt to adverse environmental conditions. Since the emergence of penicillinase-producing *Staphylococcus aureus* in the 1940s, staphylococci, enterococci and streptococci have proved themselves adept at developing or acquiring mechanisms that confer resistance to all clinically available antibacterial classes. The increasing problems of methicillin-resistant *S. aureus* and coagulase-negative staphylococci (MRSA and MRCoNS), glycopeptide-resistant enterococci and penicillin-resistant pneumococci in the 1980s, and recognition of glycopeptide-intermediate *S. aureus* in the 1990s and, most recently, of fully vancomycin-resistant isolates of *S. aureus* have emphasised our need for new anti-Gram-positive agents. Antibiotic resistance is one of the major public health concerns for the beginning of the 21st century. The pharmaceutical industry has responded with the development of oxazolidinones, lipopeptides, injectable streptogramins, ketolides, glycylcyclines, second-generation glycopeptides and novel fluoroquinolones. However, clinical use of these novel agents will cause new selective pressures and will continue to drive the development of resistance. This review describes the various antibiotic resistance mechanisms identified in isolates of staphylococci, enterococci and streptococci, including mechanisms of resistance to recently introduced anti-Gram-positive agents.

Keywords Enterococci, staphylococci, streptococci, resistance mechanisms, review

Clin Microbiol Infect 2005; 11 (Suppl. 3): 2–21

INTRODUCTION

We have been using antibiotics for <70 years but, despite our proven technological innovation, we are constantly being challenged by the ready adaptability of the bacterial pathogens that we naively sought to conquer and eradicate. This 'arms race' has resulted in bacteria that are resistant to all antibacterial classes. Antibiotic resistance remains, more than ever, a key issue for medical microbiology.

Yet, despite frequent banner headlines in the mass media, there are relatively few 'superbugs', if one defines these as bacterial pathogens resistant to all clinically available agents. From a clinical perspective, the bacteria justifying the term most fully are multi-resistant Gram-negative species (*Pseudomonas* spp., *Acinetobacter* spp. and members of the Enterobacteriaceae), which are isolated with increasing frequency both in hospitals and, even more worryingly in some instances, in the community; there is a dearth of novel agents active against such resistant isolates [1,2]. However, the epithet is more often applied to resistant strains of Gram-positive species, especially to methicillin-resistant *Staphylococcus aureus* (MRSA) and to glycopeptide- or vancomycin-resistant enterococci (GRE or VRE). The pharmaceutical industry has provided a number of recently licensed products that are active against the vast majority of such strains, and still further anti-Gram-positive agents are in development [3]. However, the spectre of resistance is never far from the horizon. There have been reports of

Corresponding author and reprint requests: N. Woodford, ARMRL, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5HT, UK
Tel: +44 20 8327 7255
Fax: +44 20 8327 6264
E-mail: neil.woodford@hpa.org.uk

resistance to novel agents, including to those licensed for clinical use since the beginning of the 21st century.

This article reviews the myriad mechanisms of resistance to diverse antibiotic classes that have been identified in resistant Gram-positive bacteria. A summary of these mechanisms is provided in Table 1.

β-LACTAM RESISTANCE

The β-lactams are the most widely used antibacterial class. Since the introduction of penicillin in the 1940s, the class has been developed and expanded to provide a continuing flow of agents with enhanced activity against bacteria resistant to preceding members.

Methicillin was introduced in the early 1960s to combat hospital strains of penicillinase-producing *S. aureus*, it being resistant to hydrolysis by these enzymes. However, resistance to methicillin was noted shortly thereafter [4]. MRSA and methicillin-resistant coagulase-negative staphylococci (MRCoNS) are all too familiar in today's hospitals [5]. In England and Wales, MRSA accounts for *c.* 40% of *S. aureus* from bacteraemias, and >70% of CoNS isolates from bacteraemias are methicillin-resistant [6]. The majority of UK MRSA isolates belong to two epidemic strains, designated EMRSA-15 and EMRSA-16 [7]. Successful clones of MRSA have been documented worldwide, and molecular epidemiology is now being used to monitor their spread [8,9].

Methicillin resistance arises following the acquisition of novel DNA, which results in production of a new penicillin-binding protein (PBP), known as PBP2′ or PBP2a, which has low binding affinity for methicillin and other currently available β-lactams (several novel anti-MRSA cephalosporins are being developed [3]). PBP2′ is the product of the *c.* 2 kb *mecA* gene, which is part of a much larger mobile genetic element, the staphylococcal chromosomal cassette *mec* (SCC*mec*). At least five different SCC*mec* types have been described, which vary in size from *c.* 20 kb (type IV) to *c.* 70 kb (type III) [10–13]. In addition to *mecA*, these complex genetic elements may also contain integrated plasmids and transposons conferring resistance to other antibiotic classes. Analysis of SCC*mec* and other staphylococcal chromosomal cassettes is providing new insights into the evolution and population biology of MRSA strains [9,11,14–17]. The origin of *mecA* remains uncertain, although a homologue has been identified in *Staphylococcus sciuri* [18].

Although penicillinase production is common in *S. aureus*, it is very rare in enterococci [19–22], and has not been reported in streptococci. Enterococci are intrinsically resistant to marketed cephalosporins because of the presence of PBP with low affinities for these agents [23]. Also, they are often tolerant of the bactericidal action of penicillins, although this may be an acquired characteristic [24]. Non-penicillinase-mediated resistance to ampicillin and penicillin is particularly associated with isolates of *Enterococcus faecium* (MIC, >8 mg/L) and also results from the presence of a low-affinity PBP, PBP5 [25]. Mutations affecting chromosomal genes, such as those encoding PBP, are not usually considered to be transferable, but mobilisation of *pbp5* by an adjacent copy of transposon Tn*5382*, which encodes VanB glycopeptide resistance (see below), has been observed from some *E. faecium* strains in the USA [26].

The rule that bacteria always develop resistance to antibiotics holds for most 'bug/drug' combinations. It is equally true that there is an exception to every biological rule. Despite over 60 years of penicillin use, no mechanism of penicillin resistance has been documented in β-haemolytic streptococci of Lancefield groups A, C or G. The reason for this absence is unknown, although isolates suspected to show resistance have been alluded to on occasion [27]. Clinical isolates of group B streptococci also remain susceptible to penicillin [6,28], although there is a report of resistance in *Streptococcus agalactiae* from dairy cows [29]. Penicillin resistance does occur in viridans streptococci and in *Streptococcus pneumoniae*. As with enterococci, the mechanism involves PBPs with reduced binding affinities, but in streptococci these are the products of mosaic genes that have arisen via inter-species transformation and recombination events [30–32]. Penicillin resistance develops in a step-wise manner with the level of resistance in a particular strain reflecting the number of PBPs affected by the mosaicism. Thus, for example, pneumococci with intermediate penicillin resistance (MIC, 0.1–1 mg/L) typically have fewer mosaic PBP species than isolates with full resistance (MIC, ≥2 mg/L). Alterations to PBPs may also cause unusual resistance phenotypes, such as isolates of pneumococci that exhibit oxacillin resistance while remaining susceptible to

Table 1. Main mechanisms of acquired antibacterial resistance in staphylococci, enterococci and streptococci

	Resistance mechanisms reported (gene)[a]		
Antibacterial class	***Staphylococcus***	***Enterococcus***	***Streptococcus***
β-Lactams	β-Lactamase (penicillinase); low-affinity PBP2′ (*mecA*) in most methicillin-resistant strains; alterations to other PBP	low-affinity PBP5 (especially in *E. faecium*); β-lactamase (pencillinase; rare)	Altered PBPs (mosaic genes); not in β-haemolytic streptococci (groups A, B, C and G)
Glycopeptides	Overproduction of D-alanyl-D-alanine (rare, GISA phenotype); teicoplanin-resistant, vancomycin-susceptible phenotype in CoNS (especially *S. epidermidis* and *S. haemolyticus*); D-alanyl-D-lactate-containing peptidoglycan precursors, *vanA* (very rare)	D-alanyl-D-lactate-containing peptidoglycan precursors, *vanA*, *vanB*, *vanD*; D-alanyl-D-serine-containing peptidoglycan precursors, *vanC*, *vanE* and *vanG*	D-alanyl-D-lactate-containing peptidoglycan precursors, *vanA*, *vanB* (rare); tolerance to vancomycin in pneumococci
Aminoglycosides	Aminoglycoside modifying enzyme: gentamicin resistance, *aac*(6′)-*aph*(2″); tobramycin/kanamycin/amikacin resistance, *ant*(4′)-*I*; kanamycin/amikacin resistance, *aph*(3′)-*III*; streptomycin resistance, *ant(6)-I*, *aph*(3″), *ant*(3″)-*I*; spectinomycin resistance, *ant(9)-I*	Aminoglycoside modifying enzyme: gentamicin resistance, *aac*(6′)-*aph*(2″) >> *aph*(2″)-*Ib*, *aph*(2″*Ic*, *aph*(2″)-*Id*; streptomycin resistance, *ant(6)-I* >> *ant*(3″)-*I*; tobramycin/kanamycin/amikacin resistance, *ant*(4′)-*I*; kanamycin/amikacin resistance, *aph*(3′)-*III*; spectinomycin resistance, *ant(9)-I*	Aminoglycoside modifying enzyme: gentamicin resistance, *aac*(6′)-*aph*(2″) (rare); streptomycin resistance, *ant*(3″)-*?*; kanamycin/amikacin resistance, *aph*(3″)-*III*; ribosomal mutations
Macrolides, lincosamides, streptogramins B and ketolides	23*S* rRNA methylase, *erm*(A), *erm*(C), *erm*(other) (MLS_B phenotype); efflux pump, *msr*(A); macrolide phosphotransferase, *mph*(C); lincosamide nucleotidyltransferase, *lnu*(A)	23*S* rRNA methylase, *erm*(B), *erm*(other) (MLS_B phenotype); efflux pump, *mef*(?), *msr*(C); lincosamide nucleotidyltransferase, *lnu*(B)	23*S* rRNA methylase, *erm*(B), *erm*(A) (MLS_B phenotype); efflux pump, *mef*(A), *mef*(E), *mre*(A); 23*S* rDNA mutations (at nucleotides A2058, A2059G, C2610 and C2611); mutations in L4 or L22 ribosomal proteins
Streptogramin combinations	Streptogramin A acetyltransferase, *vat*(A), *vat*(B) *vat*(C); streptogramin A efflux pump, *vga*(A), *vga*(B); streptogramin B lyase, *vgb*(A), *vgb*(B) (all rare)	Streptogramin A acetyltransferase, *vat*(D), *vat*(E); streptogramin B lyase, *vgb*(A) (rare)	Mutations in L22 ribosomal protein (*rplV*, rare)
Fluoroquinolones	Mutations in topoisomerase IV (*parC* > *parE*) and DNA gyrase (*gyrA* > *gyrB*); efflux pump, *norA*, other	Mutations in topoisomerase IV (*parC* > *parE*) and DNA gyrase (*gyrA* > *gyrB*); efflux pump, *emeA*, other	Mutations in topoisomerase IV (*parC* > *parE*) and DNA gyrase (*gyrA* > *gyrB*); efflux pump, *pmrA*, other
Oxazolidinones	G2576T (or T2500A) 23*S* rDNA mutation (rare)	G2576T 23*S* rDNA mutation (rare)	G2576T 23*S* rDNA mutation (rare)
Chloramphenicol	Chloramphenicol acetyltransferase, $cat_{various}$	Chloramphenicol acetyltransferase, $cat_{various}$	Chloramphenicol acetyltransferase, $cat_{various}$

Fusidic acid	Chromosomal mutations affecting EF-G, *fusA*; impermeability; inactivation; efflux pump	NA	NA
Mupirocin	Acquired isoleucyl tRNA synthetase, *mupA* (high-level resistance); chromosomal mutations, *ileS* (low-level resistance)	NA	NA
Rifampicin	Chromosomal mutations, *rpoB*	Chromosomal mutations, *rpoB*	Chromosomal mutations, *rpoB*
Tetracyclines	Efflux pump, *tet*(K), *tet*(L); ribosomal protection, *tet*(M), *tet*(O)	Ribosomal protection, *tet*(M), *tet*(O), *tet*(S); efflux pump, *tet*(K), *tet*(L); unknown mechanism, *tet*(U)	Ribosomal protection, *tet*(M), *tet*(O), *tet*(Q), *tet*(T); efflux pump, *tet*(K), *tet*(L)
Trimethoprim	Chromosomal mutations, *dfr*; acquired DHFR, *dfrA*, *dfrD*	Chromosomal mutations, *dfr*; acquired DHFR, *dfrF*	Chromosomal mutations, *dfr*

PBP, penicillin-binding protein; DHFR, dihydrofolate reductase; NA, not applicable (intrinsic resistance).
[a]See text for relevant citations.

penicillin [33], have higher MICs of amoxicillin than of penicillin [34], and have resistance to cefotaxime [35,36].

The prevalence of penicillin non-susceptible pneumococci (isolates with intermediate or full resistance) varies markedly between countries. The worldwide prevalence of fully penicillin-resistant pneumococci, estimated from over 8000 isolates from community-acquired respiratory tract infections in 26 countries, was 18.2% in 1998–2000 [37]. A rate of 35.4% (14.2% intermediate, 21.4% full resistance) among 10,000 community-acquired pneumonia isolates collected in 2001–2002 has recently been reported in the USA [38]. Among invasive isolates in the UK, data from the European Antimicrobial Resistance Surveillance System (EARSS) project indicate a decrease in penicillin non-susceptibility from 4% in 2000 to 0.5% in 2004 (http://www.earss.rivm.nl; accessed 23/09/2004). In another survey, intermediate penicillin resistance was noted in 9% of pneumococci from bacteraemia in the UK and Republic of Ireland, with no isolates showing full resistance [6].

GLYCOPEPTIDE RESISTANCE

Transferable glycopeptide resistance was first reported in 1987 and 1988 with the recognition of VanA enterococci [39–41]. Since that time, six different resistance types have been defined in enterococci; five are acquired (VanA, VanB, VanD, VanE and VanG), while the sixth, VanC, is intrinsic to *Enterococcus gallinarum* and *Enterococcus casseliflavus/Enterococcus flavescens*. GRE of all six types produce altered peptidoglycan pentapeptide precursors that terminate not in the typical D-alanyl-D-alanine, but in either of D-alanyl-D-lactate (VanA, VanB and VanD) or D-alanyl-D-serine (VanC, VanE, VanG) [42,43], which results in a much-decreased binding affinity for glycopeptides.

These substantial changes in peptidoglycan composition do not result from acquisition of a single gene; each resistance type is associated with a complex gene cluster [42]. The prototype VanA resistance cluster is found on transposon Tn*1546* [44], which carries nine genes, variously responsible for transposition (*orf1* and *orf2*), regulation and expression of resistance (*vanR-SHAX*) and accessory functions (*vanY* and *vanZ*). Similar gene clusters are found in the other

resistance types [26,42,45–53]. Phenotypically, VanA resistance is associated with high-level resistance to vancomycin (typical MIC, >128 mg/L) and, in the vast majority of strains, with cross-resistance to teicoplanin (MIC, ≥8 mg/L); some isolates with mutations in the *vanS* regulatory gene appear susceptible (or have only borderline resistance) to teicoplanin [54,55]. Other glycopeptide resistance types confer vancomycin resistance (although this may not be obvious according to all methods of susceptibility testing used in clinical laboratories), but usually spare teicoplanin, at least *in vitro*. VanA enterococci are also resistant to dalbavancin, a phase III developmental glycopeptide (MIC_{90}, 32 mg/L vs. ≤0.5 mg/L for other enterococci) [3,56]. GRE of all types usually remain susceptible to the novel glycopeptide, oritavancin, which has an evolved mechanism of action (in addition to binding D-ala-D-ala it also seems to inhibit transglycosylases) [57,58], and which is also in phase III clinical trials [3], although VanA and VanB enterococci can acquire moderate levels of resistance to this agent (MIC, ≤16 mg/L) in a single step [59].

There is homology between genes conferring acquired glycopeptide resistance in enterococci and the intrinsic resistance genes of glycopeptide-producing species and also, to some extent, in their organisation within clusters [60–63]. However, although it is likely that the producers were the ancestral source of resistance genes, the percentage G + C content of their genes differs markedly from that of enterococcal *van* genes, which argues against recent, direct escape and transfer events. In Europe, selective pressure for the emergence and dissemination of GRE (particularly VanA strains) in food production animals was exerted by use of the glycopeptide avoparcin as a growth promoter. The contribution made by this non-human reservoir of *van* resistance genes to the problem of GRE in hospitals is still hotly debated (as are similar issues for other 'bug/drug' combinations) [64,65]. The use of avoparcin was banned throughout the European Union in 1997 [66].

Currently, the prevalence of GRE in the UK is 20% in *E. faecium* and 3% in *E. faecalis* among enterococci isolated from bacteraemias [6]. Similar rates of glycopeptide resistance occur in some parts of Europe, including Greece, Ireland and Italy, but elsewhere in Europe, prevalence rates in *E. faecium* are typically <10% (EARSS project data, http://www.earss.rivm.nl; accessed 23/09/2004).

Glycopeptides retain a key role in the treatment of serious infections caused by MRSA or MRCoNS. Detection and spread of glycopeptide resistance in enterococci therefore prompted concerns that this resistance would 'escape' into staphylococci. This event was not detected until 2002, when two epidemiologically unrelated isolates of MRSA with the *vanA* gene cluster were identified in the USA [67–69]; a third unrelated *vanA* vancomycin-resistant *S. aureus* (VRSA) was reported recently, again from the USA [70]. Vancomycin MICs for these three isolates ranged from 32 to 1024 mg/L; all remained susceptible to linezolid, quinupristin–dalfopristin, and to older antibacterial agents, including rifampicin (two of three isolates), minocycline, chloramphenicol and co-trimoxazole.

Predating VRSA, but also causing public health concern, vancomycin- or glycopeptide-intermediate *S. aureus* (VISA or GISA) were first detected in Japan in 1997 [71,72], and subsequently in other countries [73–77]. These isolates display low-level vancomycin resistance (typical MIC 8–16 mg/L; categorised as 'intermediate' by the interpretative criteria of the National Committee for Clinical Laboratory Standards, hence the acronym), and decreased susceptibility to teicoplanin. Yet other *S. aureus* isolates may contain vancomycin-resistant sub-populations (so-called hetero-VISA/hetero-GISA) [78]. Analysis of data from the EARSS project (http://www.earss.rivm.nl; accessed 23/09/2004) revealed only two *S. aureus* isolates (of *c.* 65 000 isolates collected from 1999–2004 in 26 European countries) that showed decreased susceptibility to vancomycin (the level of resistance was not specified). Although this low prevalence confirms a previous report from the UK [79], the potential for poor detection of VISA/GISA in clinical laboratories [73] must be considered in international surveys.

Isolates of CoNS, particularly *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* [80], commonly show substantial resistance to teicoplanin (typical MIC, ≤64 mg/L) while remaining susceptible to vancomycin (MIC, ≤4 mg/L). The prevalence of this phenotype was estimated at 35% of CoNS from bacteraemia in the UK and Republic of Ireland [6]. Teicoplanin resistance, but vancomycin susceptibility, has also been reported rarely in *S. aureus* [80]. The prevalence of vancomycin-resistant CoNS in Europe has been estimated in several multicentre studies to be ≤0.5% [81].

The mechanism(s) of resistance in GISA, teicoplanin-resistant *S. aureus* and glycopeptide-resistant CoNS are unrelated to those of GRE or intrinsically glycopeptide-resistant Gram-positive species. The precise genetic basis for resistance remains elusive, but the GISA phenotype includes thickened cell walls and over-production of D-alanyl-D-alanine-containing peptidoglycan precursors [78,82–84]. Some GISA isolates have disrupted *agr* function (a virulence regulatory locus [85]), and an *agr*-null mutant gained heteroresistance to vancomycin and was tolerant of its bactericidal effects [86]. Mutations that inactivate *tcaA*, which encodes a putative trans-membrane protein, have also been associated with the GISA phenotype, but the mechanism has not been defined [87].

Vancomycin resistance is very uncommon among streptococci, but has been reported in an isolate of *Streptococcus bovis* from a human stool swab [88], and in isolates of *Streptococcus gallolyticus* [89,90] and *Streptococcus lutetiensis* [90] from faecal samples of veal calves. In these rare cases, resistance resulted from acquisition of gene clusters mediating the enterococcal VanB [88–90] or VanA [89] resistance mechanisms. Glycopeptide resistance has never been reported in *S. pneumoniae*, although tolerance to the bactericidal activity usually shown by vancomycin against this species has been documented [91,92].

AMINOGLYCOSIDE RESISTANCE

Although streptococci and enterococci are insusceptible to aminoglycosides (typical gentamicin MIC, 8–64 mg/L) owing to poor transport across the cytoplasmic membrane, a synergistic combination of a cell wall-active agent (penicillin or glycopeptide) with an aminoglycoside remains the treatment of choice for endocarditis caused by enterococci and viridans streptococci [93–95]. Indeed, for enterococci this is the only currently licensed therapeutic option that consistently shows bactericidal activity against susceptible isolates. Most monotherapeutic agents will not reliably kill the majority of enterococci, although the lipopeptide, daptomycin, which is licensed in the USA (albeit not for use for the treatment of endocarditis), and the second-generation glycopeptide, oritavancin (in phase III trials), are both rapidly bactericidal against many strains, including GRE (and also against MRSA, GISA and VRSA) [3]. Enterococci that present with high-level aminoglycoside resistance (gentamicin MIC, >512 mg/L; streptomycin MIC, >2000 mg/L) therefore cause therapeutic problems as the synergistic activity of the combination is abolished.

Although ribosomal mutation can result in such high levels of aminoglycoside resistance *in vitro*, especially to streptomycin [96], most resistance in the clinic is mediated by aminoglycoside modifying enzymes (AMEs). AMEs fall into three classes depending upon their biochemical effect on the aminoglycoside substrates, namely acetyltransferases (AAC), phosphotransferases (APH) and nucleotidyltransferases (ANT). Many different AMEs have been reported in staphylococci, enterococci and streptococci, and detection of identical genes in different genera provides evidence for the intergeneric spread of resistance genes.

High-level gentamicin resistance in enterococci has a prevalence in the UK of 25–50% among isolates from bacteraemias [6]. The most common mechanism underlying this phenotype is production of a bifunctional AME, AAC(6′)-APH(2″). The same enzyme is also found in gentamicin-resistant staphylococci, and has also been reported in occasional isolates of high-level gentamicin-resistant group B [97,98], group G [99] and viridans streptococci [100], and in *Aerococcus* sp. [101]. It confers resistance to all aminoglycosides available in Europe, except streptomycin. However, arbekacin, which is available in Japan, is a relatively poor substrate for AAC(6′)-APH(2″), and many gentamicin-resistant enterococci and staphylococci remain susceptible to it [102–105].

Although less prevalent than the AAC(6′)-APH(2″) bifunctional enzyme, acquired gentamicin resistance in enterococci (albeit not always at high-level) may also be conferred by APHs encoded by *aph*(2″)-*Ib* [106,107], *aph*(2″)-*Ic* [108,109], and *aph*(2″)-*Id* [110]. Arbekacin has been reported to retain useful activity against enterococci producing APH(2″)-Id [111].

High-level kanamycin resistance with amikacin resistance (the latter not always at high level) is commonly encoded by *aph*(3′)-*III* [112–114], while resistance to these combined with resistance to tobramycin (but not to gentamicin) implies presence of *ant*(4′)-*I* [114,115]; both mechanisms have been described in enterococci and staphylococci; *aph*(3′)-*III* also occurs in pneumococci [116–118]. High-level streptomycin resistance is most

commonly encoded by *ant(6)-I*, or by the ANT encoded by *ant(9)-I* and *ant*(3″)-*I* [114,119]. An ANT(3″) activity has also been reported in isolates of *Streptococcus mitis* with high-level streptomycin and kanamycin resistance [120]. Linkage of *ant(6)-I* (also known as *aadE*) and *aph*(3′)-*III* (*aphA-3*) has been demonstrated in all three genera, occasionally also including linkage to *aac*(6′)-*aph*(2″) [121,122].

RESISTANCE TO MACROLIDES, LINCOSAMIDES, STREPTOGRAMIN-B AGENTS AND KETOLIDES

The numerous mechanisms responsible for resistance to macrolides (M), lincosamides (L) and streptogramin B agents (S_B) have been the subject of recent review and nomenclature changes [123].

Cross-resistance to these three chemically diverse classes of compounds (the MLS_B phenotype) is conferred by a large number of 23*S* rRNA methylases, encoded by *erm* genes. These enzymes either mono- or dimethylate nucleotide A2058 [123], which is critical for interactions between the 23*S* rRNA and MLS_B agents [124]. The *erm* determinants may be expressed inducibly or constitutively. Macrolides (14 and 15-membered) are good inducers of resistance, but lincosamides vary in their ability to induce, being better in streptococci [123] than in staphylococci. Thus, staphylococci with inducible *erm* genes usually appear macrolide-resistant, but clindamycin-susceptible *in vitro*. Clindamycin should be avoided when considering treatment of infections caused by macrolide-resistant staphylococci because of the risk of selecting mutants with constitutive *erm* expression; these are resistant to all MLS_B agents *in vitro*. Theoretically, clindamycin remains an option if the macrolide resistance was efflux-mediated (see below), but distinguishing between Erm-mediated resistance and other macrolide resistance mechanisms is often not undertaken in clinical laboratories.

Ketolides, such as telithromycin, are poor inducers of *erm* genes and are active against many macrolide-resistant streptococci [3]. However, the extent to which they retain activity against bacteria that express *erm* genes constitutively varies [125]. This depends first on the particular *erm* determinant present (those enzymes that monomethylate A2058 result in only moderate increases in MICs of telithromycin, whereas those that dimethylate A2058 may confer high-level telithromycin resistance) and, secondly, on the efficiency of dimethylation in the particular species. Thus, constitutive Erm(B)-producing *Streptococcus pyogenes* are resistant to telithromycin, whereas constitutive Erm(B)-producing *S. pneumoniae* remain susceptible [126] most probably because the dimethylation step is more efficient in *S. pyogenes* [125].

Efflux pumps belonging to the major facilitator family [123,127,128] represent the other common mechanism of macrolide resistance; they confer resistance to macrolides only (the M phenotype) [123,129]. The *mef* genes that encode these pumps are mobile and have been found in streptococci, enterococci and several other genera [130]. *mef*(A) and *mef*(E) were originally detected in *S. pyogenes* and *S. pneumoniae*, respectively [123], although their distribution is not species-specific [131]. These genes, which share 90% DNA identity, have been considered to represent a single class, designated *mef*(A) [123], although differences in the genetic elements carrying them have led others to maintain the distinction [131].

The *erm* and *mef* genes are the most prevalent causes of macrolide resistance in Gram-positive cocci (see below). However, other mechanisms may be present: efflux pumps belonging to the ATP-binding transporter family [127,128] confer an MS_B phenotype, and are encoded by *msr*(A) alleles [which include the allele formerly called *msr*(B)] in staphylococci [123], and by *msr*(C) in *E. faecium* [132,133]; mutations in 23S rRNA or in ribosomal proteins L4/L22 have been reported in pneumococci [134–141], *S. pyogenes* [141,142], and *S. aureus* [143]; a novel efflux pump, encoded by *mre*(A), has been reported in *S. agalactiae* [144]; and macrolides may be inactivated in staphylococci that produce the Mph(C) phosphotransferase [145]. Although this latter enzyme does not confer cross-resistance to L or S_B agents, the *mph*(C) gene was found on a plasmid that also carried *msr*(A) and *erm*(Y) [145].

Specific resistance only to S_B agents in staphylococci (and rare enterococci) is conferred by the Vgb(A) and Vgb(B) lyases (see below) [146–149]. Specific resistance to lincosamides is conferred by ANT, encoded by *lnu*(A) alleles in staphylococci [formerly *lin*(A), *lin*(A′) and related genes] [123,150,151], and by *lnu*(B) [formerly *lin*(B)] in enterococci [123,152].

Efflux-mediated macrolide resistance is usually associated with lower MICs than Erm-mediated resistance. For this reason, macrolide MIC distributions often appear trimodal for bacteria in which both mechanisms occur, with peaks representing susceptible (erythromycin MIC, ≤0.5 mg/L) 'efflux-resistant' (typical erythromycin MIC, 8–16 mg/L) and 'Erm-resistant' populations (typical erythromycin MIC, ≥256 mg/L). This is illustrated, for example, by α-, β- and non-haemolytic streptococci from UK bacteraemias [6], and by pneumococci from PROTEKT US [38].

The prevalence of macrolide resistance among pneumococci exceeds rates of penicillin resistance. In the PROTEKT US survey, 28% of *c.* 10 000 isolates of *S. pneumoniae* collected in 2001–2002 were resistant to erythromycin (MIC, ≥1 mg/L), and <1% showed reduced susceptibility to the ketolide telithromycin; 70% of macrolide-resistant isolates were susceptible to clindamycin [38]. The mechanisms detected were: *mef*(A) only, 68.7%; *erm*(B) only, 16.8%; *mef*(A) + *erm*(B), 12.2%; *erm*(A), 0.2%; and unknown, 2% [153]. The worldwide prevalence of macrolide resistance in pneumococci, estimated from isolates collected 1998–2000, was 24.6% [37]. In the UK, 17% of pneumococci from bacteraemias were resistant to erythromycin [6], as were 10% of isolates from community-acquired respiratory infections [154].

Erythromycin resistance was found in 5.7% of *S. pyogenes* isolates from PROTEKT US [38], and in 10% of β-haemolytic streptococci (of groups A, B, C and G) from UK bacteraemias [6]. In the UK survey, 7% of β-haemolytic streptococci showed low-level (probable Mef-mediated) macrolide resistance; the remaining macrolide-resistant isolates showed high-level (probable Erm-mediated) resistance [6]. Similarly, 24% of α- and non-haemolytic streptococci from UK bacteraemias were resistant to erythromycin; 17% showed low-level (probable Mef-mediated) resistance; and 7% isolates showed high-level (probable Erm-mediated) resistance [6].

RESISTANCE TO STREPTOGRAMIN COMBINATIONS

A pristinamycin combination has been available as an anti-staphylococcal agent for many years in some European countries, for example, France [155], but quinupristin/dalfopristin, which was licensed in the late 1990s, represented the first water-soluble streptogramin combination developed for injection. Quinupristin/dalfopristin consists of a synergistic mixture of a streptogramin A (S_A, dalfopristin) and a streptogramin B (S_B, quinupristin). These structurally distinct molecules act on different sites of the 50*S* ribosomal sub-unit and, given together, are bactericidal against many Gram-positive bacteria [156]. The combination is active *in vitro* against the vast majority of staphylococci and against many *E. faecium*, including glycopeptide-resistant strains. However, it has no activity against most *E. faecalis* strains, because of its apparent efflux via the Lsa pump, which appears to be intrinsic to the species [157–159], and its activity against other enterococcal species may be inferior to that against *E. faecium* [160].

Resistance to streptogramin combinations requires resistance specifically to the S_A component, but is augmented by the presence of mechanisms conferring S_B resistance [161]. S_A resistance can be mediated by several mechanisms: specific acetyl transferases encoded by *vat*(A), *vat*(B) and *vat*(C) have been characterised in staphylococci [147,162,163], and by *vat*(D) and *vat*(E) (formerly *satA* and *satG*) in *E. faecium* [164,165]; also, S_A efflux pumps encoded by *vga*(A) and *vga*(B) have been identified in staphylococci [166,167]. Cross-resistance encompassing S_B compounds is commonly mediated by *erm* determinants (see above), but specific resistance to S_B agents is mediated by lyases, which inactivate the compounds and which are encoded by *vgb*(A) and *vgb*(B) [146–148]. Although these genes occur mainly in staphylococci, *vgb*(A) has been identified in occasional isolates of enterococci [149]. The *vat*(A–E) genes are often plasmid-mediated in staphylococci and *E. faecium*, which permits ready transfer between strains. Genetic linkage of multiple streptogramin resistance mechanisms on individual plasmids has been reported [147,168,169], as has linkage to genes conferring resistance to other antimicrobial classes [170–173]. In addition to acquired resistance mechanisms, low-level resistance to quinupristin/dalfopristin (MIC, 4 mg/L) arising from mutations in *rplV*, which encodes ribosomal protein L22, has been reported in a clinical isolate of *S. aureus* [143] and in two clinical isolates of *S. pneumoniae* [136].

There is concern that dissemination of *vat*(D) and *vat*(E) in *E. faecium* has been selected by use of

virginiamycin (another streptogramin combination) as a growth promoter in food production animals. These genes are found relatively readily in enterococci isolated from treated animals and retail meat [174–180], but have also been recovered from isolates from human clinical specimens or faeces, in some instances prior to licensing of quinupristin/dalfopristin [173,181,182], which provides circumstantial evidence for spread from a pre-existing reservoir of resistance. However, clinical isolates of *E. faecium* resistant to streptogramin combinations remain rare, which probably reflects low usage of quinupristin/dalfopristin. Low-level quinupristin/dalfopristin resistance (MIC 4–8 mg/L) may also be observed in clinical isolates of *E. faecium* that lack defined resistance determinants.

FLUOROQUINOLONE RESISTANCE

The fluoroquinolones target DNA gyrase (topoisomerase II) and topoisomerase IV, which are essential for the DNA supercoiling of bacterial DNA and any process that requires it (e.g., DNA replication, transcription, etc.). Resistance to fluoroquinolones, which are synthetic, arises via mutational target modification or efflux; drug inactivation has not been reported in bacteria. Earlier analogues, such as ciprofloxacin, tend to be affected more than newer generation fluoroquinolones; as with many other antibiotic classes, the pharmaceutical industry has provided a multitude of novel quinolones, which extend the spectrum of the class, particularly against Gram-positive cocci [3,183].

In contrast with mutational resistance to many other antibiotics, acquisition of a single target-modifying mutation does not usually confer significant fluoroquinolone resistance. Rather, resistance is a cumulative process, with increasing numbers of mutations generally correlating with increasing MICs of fluoroquinolones. Both the DNA gyrase and topoisomerase IV targets consist of sub-units. These are encoded by *gyrA* and *gyrB* (for DNA gyrase), and by *parC* and *parE* (for topoisomerase IV). Mutations that confer fluoroquinolone resistance arise in *gyrA* and *parC* (changes in *gyrB* and *parE* also occur, but are less common), and are usually clustered in specific areas of the genes, known as the quinolone resistance determining regions. The quinolone resistance determining regions centre around the codons that encode residues serine-81 of GyrA and serine-79 of ParC. The mechanisms and epidemiology of fluoroquinolone resistance in Gram-positive cocci have been reviewed recently [184–186]. Low-level resistance results from mutations in either GyrA or ParC, whereas high-level resistance is associated with mutations in both proteins. The gene affected in first-step mutational events depends on the primary lethal target of the fluoroquinolone under study; some analogues target GyrA preferentially, others ParC, and yet others have 'dual action' [3,183]. Mutational resistance to fluoroquinolones has been reported extensively in staphylococci and pneumococci (for reviews, see [185,186]). It also occurs in enterococci [187–192], and has been reported in occasional isolates of *S. pyogenes* [193–195].

Efflux-mediated resistance involves multi-drug efflux pumps (MDRs) belonging to the major facilitator family [123,127,128] rather than fluoroquinolone-specific channels, so other antibiotic classes tend also to be affected. These pumps include NorA (and others) in *S. aureus* [196–201] and PmrA (and others) in pneumococci [202–207]. Also, interrogation of the *E. faecalis* genome led to characterisation of *emeA*, which encodes a NorA homologue, and to the recognition of many other putative MDRs in this species [208,209].

A novel fluoroquinolone resistance mechanism, involving decreased expression of topoisomerase IV, was recently identified in a laboratory-generated *S. aureus* mutant that showed low-level ciprofloxacin resistance (the MIC rose 4- to 8-fold, from 0.125–0.25 to 1 mg/L) [210].

OXAZOLIDINONE RESISTANCE

Linezolid was the first oxazolidinone to be licensed [211], although other members of this novel class of antibacterial agents are in development [3]. Oxazolidinones bind to the 50*S* ribosomal sub-unit [212] and prevent protein synthesis by inhibiting formation of the 70*S* ribosomal initiation complex. Cross-linking studies have revealed that linezolid binds with the conserved nucleotide A2602 in the 23*S* rRNA, with ribosomal protein L27, and with ribosome-associated LepA (a protein with homology to translation factors), indicating that peptidyl transferase is the primary target for the agent [213].

The oxazolidinones are synthetic agents and there is no pre-existing reservoir of resistance. Resistance arises, not from the acquisition of

genes, but by mutation in chromosomal genes encoding 23*S* rRNA. A number of mutations affecting the peptidyl transferase domain of the 23*S* rRNA have been shown to confer linezolid resistance in laboratory-generated mutants [214]. Mutation of nucleotide G2576 → T (with reference to the *Escherichia coli* numbering of GenBank AF053964) is the most widely reported mutation among linezolid-resistant clinical isolates (MIC, >4 mg/L), having been found in enterococci [215–218], *S. aureus* [219–221], and also in single isolates of *S. epidermidis* and *Streptococcus oralis* (the latter isolates had additional mutations in their 23*S* rRNA, but the contribution of these to linezolid resistance, if any, was not ascertained) [222]; a T2500A mutation was recently reported in a clinical isolate of *S. aureus* [223].

Unusually for bacterial genes, the rDNA genes of most species are present in multiple copies. Oxazolidinone resistance probably arises via a two-step process, an initial mutational event affecting one gene copy, followed by intragenomic recombination (also known as gene conversion) events to distribute the mutation to sufficient rDNA alleles to confer phenotypic resistance. In support of this, the MIC of linezolid for resistant enterococci correlates with the copy number of rDNA alleles possessing the mutation [224–226], and resistance arose less readily in a recombination-deficient strain of *E. faecalis* [227].

To date, linezolid resistance has emerged only rarely and usually during treatment, although there are exceptions to both of these generalisations [228,229]. As resistance is mutational and not transferable between strains, we must be concerned about the potential for spread of the linezolid-resistant strains. Thus, infection control is the major issue when linezolid-resistant enterococci or staphylococci are isolated. A nosocomial cluster of infections caused by linezolid-resistant enterococci affecting eight patients on an oncology ward in the USA has been reported [230], but there are no reports of cross-infection with linezolid-resistant MRSA. However, unfortunately, the prevalence of MRSA in many countries stands as poor testament to the effectiveness of infection control in many hospitals. In the UK, the emergence during therapy of linezolid-resistant isolates of EMRSA-15 [220] is a worrying development given the predilection of this strain for nosocomial spread [6,7].

RESISTANCE TO OTHER AGENTS

Chloramphenicol

Most resistance to chloramphenicol in Gram-positive cocci is mediated by chloramphenicol acetyl transferases, which are encoded by plasmid-mediated or chromosomally integrated *cat* genes. Many *cat* variants have been identified, and they fall into distinct classes on the basis of hybridisation data and their sequence [231]. Several are shared by staphylococci, enterococci and streptococci. For example, the staphylococcal cat_{pC194} determinant [232] is associated with conjugative transposon Tn*1545* in pneumococci [116,233,234]; it is also found in other streptococci and in enterococci [231]. The cat_{pC221} and cat_{pSCS7} determinants are also from staphylococci, but have been detected in enterococci and streptococci [231,232,235].

Fusidic acid

Resistance to fusidic acid (an inhibitor of elongation factor G) arises in staphylococci by various mechanisms. Fusidic acid monotherapy is usually avoided in hospitals because of the likelihood of selection of resistant mutants [236]; these usually have alterations in *fusA*, which encodes EF-G [237–240]. In the UK, increased (mainly topical) use of the agent in the community has been associated with rising resistance rates in *S. aureus* [241]. Other fusidic acid resistance mechanisms occur in staphylococci, but few have been precisely defined; they include impermeability and efflux, and some are plasmid-mediated [239,242,243]. Inactivation of fusidic acid by some CAT enzymes has also been reported, but the clinical relevance of this to staphylococci that are fusidic acid-susceptible, chloramphenicol-resistant *in vitro* is unknown [239].

Mupirocin

Mupirocin is a topical anti-staphylococcal agent used, for example, to eradicate MRSA carriage. It inhibits isoleucyl tRNA synthetase, thereby stopping protein synthesis. Staphylococci can develop resistance to mupirocin by two mechanisms: chromosomal mutations in the chromosomal *ileS* gene give rise to low levels of resistance (MIC, 8–128 mg/L) [244,245], which are not considered to be clinically significant because topical application gives local concentrations far in excess of

the MIC; acquisition of a second, resistant isoleucyl tRNA synthetase (encoded by *mupA*, which is often plasmid-mediated) by-passes the action of mupirocin and gives clinically significant, high-level resistance (MIC, ≥256 mg/L) [244,246–248].

Rifampicin

Rifampicin resistance arises readily via chromosomal mutations in *rpoB*, which encodes the β-subunit of RNA polymerase. This mechanism has been described in staphylococci [249–251], enterococci [252] and streptococci [253]. *Nocardia* and related bacteria are able to inactivate rifampicin via a number of mechanisms [254]. These mechanisms have not been reported in Gram-positive cocci, although *arr-2* (encoding a rifampicin ADP ribosyltransferase) has been detected as an integron-borne resistance cassette in Gram-negative bacteria [255].

Tetracyclines

Tetracycline resistance in Gram-positive cocci is mediated by two main mechanisms, efflux pumps and ribosomal protection systems [256,257]. Typically, the efflux pumps confer resistance (MIC, >8 mg/L) to tetracycline and doxycycline, but not to minocycline, whereas the ribosomal protection systems confer resistance also to minocycline. The specific mechanisms described are: for staphylococci, the Tet(K) and Tet(L) efflux pumps, plus the Tet(M) and Tet(O) ribosomal protection systems [256,258]; for enterococci, Tet(K), Tet(L), Tet(M), Tet(O), plus the Tet(S) ribosomal protection system, and the *tet*(U) deteminant, which confers resistance by an undefined mechanism [256,259]; for streptococci, Tet(K), Tet(L), Tet(M), Tet(O), plus the Tet(Q) and Tet(T) ribosomal protection systems [256]. Arguably, *tet*(M), associated with the conjugative transposon Tn*916*, is one of the most successful of all antibiotic resistance determinants.

Mechanisms conferring resistance to the glycylcycline tigecycline have not yet been identified in Gram-positive cocci. Bacteria resistant to earlier tetracycline analogues by efflux or ribosomal protection mechanisms usually remain susceptible to tigecycline [3]. However, mutations in the Tet(A) and Tet(B) efflux pumps of Gram-negative bacteria can confer resistance to tigecycline [256,260,261], and the AcrAB pump is responsible for the intrinsic reduced susceptibility of *Proteus mirabilis* to tigecycline [262]. By analogy, it seems possible that efflux-mediated reduced susceptibility will eventually emerge in Gram-positives.

Trimethoprim

In pneumococci, resistance to trimethoprim reflects mutations in the chromosomal *dfr* gene encoding dihydrofolate reductase (DHFR) [233]. As with the PBP changes that mediate β-lactam resistance in this species, interspecies recombination with other streptococci is considered important for the diversification of pneumococcal *dfr* [263]. Chromosomal *dfr* mutations also occur in staphylococci [264] and enterococci [265].

Plasmid-mediated resistance in staphylococci is associated with acquired DHFR S1 and S2, encoded by *dfrA* and *dfrD* respectively [266,267]. Both probably represent the 'escape' to plasmids of chromosomal *dfr* genes from CoNS species. Transferable, plasmid-mediated trimethoprim resistance has been observed in enterococci, although the mechanism was not elucidated [268]. An acquired, chromosomally located DHFR (encoded by *dfrF*), which conferred high-level trimethoprim resistance (MIC, >1024 mg/L) has been characterised in a strain of *E. faecalis* [265]. When sought, the staphylococcal *dfrA* determinant has not been found in enterococci [269].

CONCLUDING REMARKS

Bacteria have proved themselves able to develop or acquire resistance to every antibiotic class used against them. This review has summarised the myriad mechanisms encountered in Gram-positive cocci, but the mechanisms of Gram-negative bacteria are equally diverse and, arguably, present far greater clinical challenges at the present time. Anti-Gram-positive agents such as linezolid and quinupristin/dalfopristin retain good activity against the majority of MRSA, GRE and streptococci, but resistance has been reported even to these. We need to develop agents with enhanced modes of action, such as the second-generation glycopeptide oritavancin or agents with novel mechanisms of action, such as the lipopeptide daptomycin [3], while bearing in mind the fact that any antibiotic has only a limited period of 'virginity' before resistance emerges. We must implement strategies that maximise these periods and maintain the efficacy of new agents.

REFERENCES

1. Livermore DM. The threat from the pink corner. *Ann Med* 2003; **35:** 226–234.
2. Woodford N, Ward ME, Kaufmann ME *et al.* Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β-lactamases in the UK. *J Antimicrob Chemother* 2004; **54:** 735–743.
3. Woodford N. Novel agents for the treatment of resistant Gram-positive infections. *Expert Opin Investig Drugs* 2003; **12:** 117–137.
4. Jevons MP. ''Celbenin''-resistant staphylococci. *Brit Med J* 1961; **1:** 124–125.
5. Livermore DM. Antibiotic resistance in staphylococci. *Int J Antimicrob Agents* 2000; **16**(suppl 1)**:** 3–10.
6. Reynolds R, Potz N, Colman M, Williams A, Livermore D, MacGowan A. Antimicrobial susceptibility of the pathogens of bacteraemia in the UK and Ireland 2001–2002: the BSAC Bacteraemia Resistance Surveillance Programme. *J Antimicrob Chemother* 2004; **53:** 1018–1032.
7. Johnson AP, Aucken HM, Cavendish S *et al.* Dominance of EMRSA-15 and –16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *J Antimicrob Chemother* 2001; **48:** 143–144.
8. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; **38:** 1008–1015.
9. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci USA* 2002; **99:** 7687–7692.
10. Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. *Trends Microbiol* 2001; **9:** 486–493.
11. Oliveira DC, Tomasz A, De Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis* 2002; **2:** 180–189.
12. Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. *Antimicrob Agents Chemother* 2004; **48:** 2637–2651.
13. Huletsky A, Giroux R, Rossbach V *et al.* New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* 2004; **42:** 1875–1884.
14. Oliveira DC, Tomasz A, De Lencastre H. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. *Microb Drug Resist* 2001; **7:** 349–361.
15. Robinson DA, Enright MC. Evolution of *Staphylococcus aureus* by large chromosomal replacements. *J Bacteriol* 2004; **186:** 1060–1064.
16. Holden MT, Feil EJ, Lindsay JA *et al.* Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. *Proc Natl Acad Sci USA* 2004; **101:** 9786–9791.
17. Mongkolrattanothai K, Boyle S, Murphy TV, Daum RS. Novel non-*mecA*-containing staphylococcal chromosomal cassette composite island containing *pbp4* and *tagF* genes in a commensal staphylococcal species: a possible reservoir for antibiotic resistance islands in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2004; **48:** 1823–1836.
18. Wu S, Piscitelli C, De Lancastre H, Tomasz A. Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of *mecA* from a methicillin-susceptible strain of *Staphylococcus sciuri*. *Microb Drug Resist* 1996; **2:** 435–441.
19. Murray BE. β-lactamase-producing enterococci. *Antimicrob Agents Chemother* 1992; **36:** 2355–2359.
20. Murray BE, Lopardo HA, Rubeglio E, Frosolono M, Singh KV. Intrahospital spread of a single gentamicin-resistant, β-lactamase-producing strain of *Enterococcus faecalis* in Argentina. *Antimicrob Agents Chemother* 1992; **36:** 230–232.
21. Murray BE, Singh KV, Markowitz SM *et al.* Evidence for clonal spread of a single strain of β-lactamase-producing *Enterococcus* (*Streptococcus*) *faecalis* to six hospitals in five states. *J Infect Dis* 1991; **163:** 780–785.
22. Wanger AR, Murray BE. Comparison of enterococcal and staphylococcal β-lactamase plasmids. *J Infect Dis* 1990; **161:** 54–58.
23. Arbeloa A, Segal H, Hugonnet JE *et al.* Role of class A penicillin-binding proteins in PBP5-mediated β-lactam resistance in *Enterococcus faecalis*. *J Bacteriol* 2004; **186:** 1221–1228.
24. Moellering RCJ. The enterococcus: a classic example of the impact of antimicrobial resistance on therapeutic options. *J Antimicrob Chemother* 1991; **28:** 1–12.
25. Sauvage E, Kerff F, Fonze E *et al.* The 2.4-A crystal structure of the penicillin-resistant penicillin-binding protein PBP5fm from *Enterococcus faecium* in complex with benzylpenicillin. *Cell Mol Life Sci* 2002; **59:** 1223–1232.
26. Carias LL, Rudin SD, Donskey CJ, Rice LB. Genetic linkage and cotransfer of a novel, *vanB*-containing transposon (Tn*5382*) and a low-affinity penicillin-binding protein 5 gene in a clinical vancomycin-resistant *Enterococcus faecium* isolate. *J Bacteriol* 1998; **180:** 4426–4434.
27. Baquero F. Gram-positive resistance: challenge for the development of new antibiotics. *J Antimicrob Chemother* 1997; **39**(suppl A)**:** 1–6.
28. Weisner AM, Johnson AP, Lamagni TL *et al.* Characterization of group B streptococci recovered from infants with invasive disease in England and Wales. *Clin Infect Dis* 2004; **38:** 1203–1208.
29. Berghash SR, Dunny GM. Emergence of a multiple β-lactam-resistance phenotype in group B streptococci of bovine origin. *J Infect Dis* 1985; **151:** 494–500.
30. Dowson CG, Hutchison A, Woodford N, Johnson AP, George RC, Spratt BG. Penicillin-resistant viridans streptococci have obtained altered penicillin-binding protein genes from penicillin-resistant strains of *Streptococcus pneumoniae*. *Proc Natl Acad Sci USA* 1990; **87:** 5858–5862.
31. Coffey TJ, Dowson CG, Daniels M, Spratt BG. Horizontal spread of an altered penicillin-binding protein 2B gene between *Streptococcus pneumoniae* and *Streptococcus oralis*. *FEMS Microbiol Lett* 1993; **110:** 335–339.
32. Dowson CG, Coffey TJ, Kell C, Whiley RA. Evolution of penicillin resistance in *Streptococcus pneumoniae*; the role

of *Streptococcus mitis* in the formation of a low affinity PBP2B in *S. pneumoniae*. *Mol Microbiol* 1993; **9:** 635–643.
33. Dowson CG, Johnson AP, Cercenado E, George RC. Genetics of oxacillin resistance in clinical isolates of *Streptococcus pneumoniae* that are oxacillin resistant and penicillin susceptible. *Antimicrob Agents Chemother* 1994; **38:** 49–53.
34. Kosowska K, Jacobs MR, Bajaksouzian S, Koeth L, Appelbaum PC. Alterations of penicillin-binding proteins 1A, 2X, and 2B in *Streptococcus pneumoniae* isolates for which amoxicillin MICs are higher than penicillin MICs. *Antimicrob Agents Chemother* 2004; **48:** 4020–4022.
35. Munoz R, Dowson CG, Daniels M *et al.* Genetics of resistance to third-generation cephalosporins in clinical isolates of *Streptococcus pneumoniae*. *Mol Microbiol* 1992; **6:** 2461–2465.
36. Coffey TJ, Daniels M, McDougal LK, Dowson CG, Tenover FC, Spratt BG. Genetic analysis of clinical isolates of *Streptococcus pneumoniae* with high-level resistance to expanded-spectrum cephalosporins. *Antimicrob Agents Chemother* 1995; **39:** 1306–1313.
37. Jacobs MR, Felmingham D, Appelbaum PC, Gruneberg RN. The Alexander Project 1998–2000: susceptibility of pathogens isolated from community-acquired respiratory tract infection to commonly used antimicrobial agents. *J Antimicrob Chemother* 2003; **52:** 229–246.
38. Brown SD, Rybak MJ. Antimicrobial susceptibility of *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Haemophilus influenzae* collected from patients across the USA, in 2001–2002, as part of the PROTEKT US study. *J Antimicrob Chemother* 2004; **54**(suppl 1)**:** i7–i15.
39. Leclercq R, Derlot E, Duval J, Courvalin P. Strains of *Enterococcus faecium* highly resistant to vancomycin and teicoplanin. In: *Abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy*. New York, NY: American Society for Microbiology, 1987; 275.
40. Uttley AHC, Collins CH, Naidoo J, George RC. Vancomycin-resistant enterococci. *Lancet* 1988; **I:** 57–58.
41. Leclercq R, Derlot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N Engl J Med* 1988; **319:** 157–161.
42. Woodford N. Epidemiology of the genetic elements responsible for acquired glycopeptide resistance in enterococci. *Microb Drug Resist* 2001; **7:** 229–236.
43. Reynolds PE, Courvalin P. Vancomycin resistance in enterococci due to synthesis of precursors terminating in D-alanyl-D-serine. *Antimicrob Agents Chemother* 2005; **49:** 21–25.
44. Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn*1546*, a Tn*3*-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J Bacteriol* 1993; **175:** 117–127.
45. Quintiliani RJ, Courvalin P. Characterization of Tn*1547*, a composite transposon flanked by IS*16* and IS*256*-like elements, that confers vancomycin resistance in *Enterococcus faecalis* BM4281. *Gene* 1996; **172:** 1–8.
46. Casadewall B, Courvalin P. Characterization of the *vanD* glycopeptide resistance gene cluster from *Enterococcus faecium* BM4339. *J Bacteriol* 1999; **181:** 3644–3648.
47. Dalla Costa LM, Reynolds PE, Souza HA, Souza DC, Palepou MF, Woodford N. Characterization of a divergent *vanD*-type resistance element from the first glycopeptide-resistant strain of *Enterococcus faecium* isolated in Brazil. *Antimicrob Agents Chemother* 2000; **44:** 3444–3446.
48. Boyd DA, Kibsey P, Roscoe D, Mulvey MR. *Enterococcus faecium* N03–0072 carries a new VanD-type vancomycin resistance determinant: characterization of the VanD5 operon. *J Antimicrob Chemother* 2004; **54:** 680–683.
49. Patino LA, Courvalin P, Perichon B. *vanE* gene cluster of vancomycin-resistant *Enterococcus faecalis* BM4405. *J Bacteriol* 2002; **184:** 6457–6464.
50. Boyd DA, Cabral T, Van Caeseele P, Wylie J, Mulvey MR. Molecular characterization of the *vanE* gene cluster in vancomycin-resistant *Enterococcus faecalis* N00–410 isolated in Canada. *Antimicrob Agents Chemother* 2002; **46:** 1977–1979.
51. McKessar SJ, Berry AM, Bell JM, Turnidge JD, Paton JC. Genetic characterization of *vanG*, a novel vancomycin resistance locus in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2000; **44:** 3224–3228.
52. Depardieu F, Bonora MG, Reynolds PE, Courvalin P. The *vanG* glycopeptide resistance operon from *Enterococcus faecalis* revisited. *Mol Microbiol* 2003; **50:** 931–948.
53. Arias CA, Courvalin P, Reynolds PE. *vanC* cluster of vancomycin-resistant *Enterococcus gallinarum* BM4174. *Antimicrob Agents Chemother* 2000; **44:** 1660–1666.
54. Hashimoto Y, Tanimoto K, Ozawa Y, Murata T, Ike Y. Amino acid substitutions in the VanS sensor of the VanA-type vancomycin-resistant *Enterococcus* strains result in high-level vancomycin resistance and low-level teicoplanin resistance. *FEMS Microbiol Lett* 2000; **185:** 247–254.
55. Lauderdale TL, McDonald LC, Shiau YR *et al.* Vancomycin-resistant enterococci from humans and retail chickens in Taiwan with unique VanB phenotype-*vanA* genotype incongruence. *Antimicrob Agents Chemother* 2002; **46:** 525–527.
56. Jones RN, Biedenbach DJ, Johnson DM, Pfaller MA. *In vitro* evaluation of BI 397, a novel glycopeptide antimicrobial agent. *J Chemother* 2001; **13:** 244–254.
57. Ge M, Chen Z, Onishi HR *et al.* Vancomycin derivatives that inhibit peptidoglycan biosynthesis without binding D-Ala-D-Ala. *Science* 1999; **284:** 507–511.
58. Walsh C. Deconstructing vancomycin. *Science* 1999; **284:** 442–443.
59. Arthur M, Depardieu F, Reynolds P, Courvalin P. Moderate-level resistance to glycopeptide LY333328 mediated by genes of the *vanA* and *vanB* clusters in enterococci. *Antimicrob Agents Chemother* 1999; **43:** 1875–1880.
60. Marshall CG, Broadhead G, Leskiw BK, Wright GD. D-Ala-D-Ala ligases from glycopeptide antibiotic-producing organisms are highly homologous to the enterococcal vancomycin-resistance ligases VanA and VanB. *Proc Natl Acad Sci USA* 1997; **94:** 6480–6483.
61. Marshall CG, Lessard IAD, Park I-S, Wright GD. Glycopeptide antibiotic resistance genes and glycopeptide-producing organisms. *Antimicrob Agents Chemother* 1998; **42:** 2215–2220.
62. Pootoolal J, Thomas MG, Marshall CG *et al.* Assembling the glycopeptide antibiotic scaffold: the biosynthesis of A47934 from *Streptomyces toyocaensis* NRRL15009. *Proc Natl Acad Sci USA* 2002; **99:** 8962–8967.
63. Hong HJ, Hutchings MI, Neu JM, Wright GD, Paget MS, Buttner MJ. Characterization of an inducible vancomycin resistance system in *Streptomyces coelicolor* reveals a novel

gene (*vanK*) required for drug resistance. *Mol Microbiol* 2004; **52:** 1107–1121.
64. Phillips I, Casewell M, Cox T *et al.* Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J Antimicrob Chemother* 2004; **53:** 28–52.
65. Turnidge J. Antibiotic use in animals – prejudices, perceptions and realities. *J Antimicrob Chemother* 2004; **53:** 26–27.
66. Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother* 2003; **52:** 159–161.
67. Sievert DM, Boulton ML, Stoltman G *et al. Staphylococcus aureus* resistant to vancomycin – United States, 2002. *MMWR* 2002; **51:** 565–567.
68. Miller D, Urdaneta V, Weltman A, Park S. Vancomycin-resistant *Staphylococcus aureus* – Pennsylvania, 2002. *MMWR* 2002; **51:** 902.
69. Clark NC, Weigel LM, Patel JB, Tenover FC. Comparison of Tn*1546*-like elements in vancomycin-resistant *Staphylococcus aureus* isolates from Michigan and Pennsylvania. *Antimicrob Agents Chemother* 2005; **49:** 470–472.
70. CDC. Vancomycin-resistant *Staphylococcus aureus*–New York, 2004. *MMWR* 2004; **53:** 322–323.
71. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997; **40:** 135–136.
72. Hiramatsu K, Aritaka N, Hanaki H *et al.* Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet* 1997; **350:** 1670–1673.
73. Tenover FC, Lancaster MV, Hill BC *et al.* Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. *J Clin Microbiol* 1998; **36:** 1020–1027.
74. Kim MN, Pai CH, Woo JH, Ryu JS, Hiramatsu K. Vancomycin-intermediate *Staphylococcus aureus* in Korea. *J Clin Microbiol* 2000; **38:** 3879–3881.
75. Oliveira GA, Dell'Aquila AM, Masiero RL *et al*. Isolation in Brazil of nosocomial *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Infect Control Hosp Epidemiol* 2001; **22:** 443–448.
76. Werner G, Cuny C, Schmitz FJ, Witte W. Methicillin-resistant, quinupristin–dalfopristin-resistant *Staphylococcus aureus* with reduced sensitivity to glycopeptides. *J Clin Microbiol* 2001; **39:** 3586–3590.
77. Howe RA, Monk A, Wootton M, Walsh TR, Enright MC. Vancomycin susceptibility within methicillin-resistant *Staphylococcus aureus* lineages. *Emerg Infect Dis* 2004; **10:** 855–857.
78. Hiramatsu K. Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. *Lancet Infect Dis* 2001; **1:** 147–155.
79. Aucken HM, Warner M, Ganner M *et al*. Twenty months of screening for glycopeptide-intermediate *Staphylococcus aureus*. *J Antimicrob Chemother* 2000; **46:** 639–640.
80. Woodford N, Johnson AP, Morrison D, Speller DCE. Current perspectives on glycopeptide resistance. *Clin Microbiol Rev* 1995; **8:** 585–615.
81. Srinivasan A, Dick JD, Perl TM. Vancomycin resistance in staphylococci. *Clin Microbiol Rev* 2002; **15:** 430–438.
82. Hanaki H, Labischinski H, Inaba Y, Kondo N, Murakami H, Hiramatsu K. Increase in glutamine-non-amidated muropeptides in the peptidoglycan of vancomycin-resistant *Staphylococcus aureus* strain Mu50. *J Antimicrob Chemother* 1998; **42:** 315–320.
83. Cui L, Murakami H, Kuwahara-Arai K, Hanaki H, Hiramatsu K. Contribution of a thickened cell wall and its glutamine nonamidated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50. *Antimicrob Agents Chemother* 2000; **44:** 2276–2285.
84. Cui L, Ma X, Sato K *et al.* Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 2003; **41:** 5–14.
85. Novick RP, Muir TW. Virulence gene regulation by peptides in staphylococci and other Gram-positive bacteria. *Curr Opin Microbiol* 1999; **2:** 40–45.
86. Sakoulas G, Eliopoulos GM, Moellering RC Jr *et al.* Accessory gene regulator (*agr*) locus in geographically diverse *Staphylococcus aureus* isolates with reduced susceptibility to vancomycin. *Antimicrob Agents Chemother* 2002; **46:** 1492–1502.
87. Maki H, McCallum N, Bischoff M, Wada A, Berger-Bachi B. *tcaA* inactivation increases glycopeptide resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2004; **48:** 1953–1959.
88. Poyart C, Pierre C, Quesne G, Pron B, Berche P, Trieu-Cuot P. Emergence of vancomycin resistance in the genus *Streptococcus*: characterization of a vanB transferable determinant in *Streptococcus bovis*. *Antimicrob Agents Chemother* 1997; **41:** 24–29.
89. Mevius D, Devriese L, Butaye P, Vandamme P, Verschure M, Veldman R. Isolation of glycopeptide resistant *Streptococcus gallolyticus* strains with *vanA*, *vanB*, and both *vanA* and *vanB* genotypes from faecal samples of veal calves in The Netherlands. *J Antimicrob Chemother* 1998; **42:** 275–276.
90. Dahl KH, Sundsfjord A. Transferable *vanB2* Tn*5382*-containing elements in fecal streptococcal strains from veal calves. *Antimicrob Agents Chemother* 2003; **47:** 2579–2583.
91. Novak R, Henriques B, Charpentier E, Normark S, Tuomanen E. Emergence of vancomycin tolerance in *Streptococcus pneumoniae*. *Nature* 1999; **399:** 590–593.
92. Henriques NB, Novak R, Ortqvist A, Kallenius G, Tuomanen E, Normark S. Clinical isolates of *Streptococcus pneumoniae* that exhibit tolerance of vancomycin. *Clin Infect Dis* 2001; **32:** 552–558.
93. Working Party of the British Society for Antimicrobial Chemotherapy. Antibiotic treatment of streptococcal, enterococcal, and staphylococcal endocarditis. *Heart* 1998; **79:** 207–210.
94. Delahaye F, Hoen B, McFadden E, Roth O, de Gevigney G. Treatment and prevention of infective endocarditis. *Expert Opin Pharmacother* 2002; **3:** 131–145.
95. Graham JC, Gould FK. Role of aminoglycosides in the treatment of bacterial endocarditis. *J Antimicrob Chemother* 2002; **49:** 437–444.
96. Salles C, Creancier L, Claverys JP, Mejean V. The high level streptomycin resistance gene from *Streptococcus pneumoniae* is a homologue of the ribosomal protein S12 gene from *Escherichia coli*. *Nucleic Acids Res* 1992; **20:** 6103.
97. Buu-Hoi A, Le Bouguenec C, Horaud T. High-level chromosomal gentamicin resistance in *Streptococcus aga-*

lactiae (group B). *Antimicrob Agents Chemother* 1990; **34:** 985–988.
98. Kaufhold A, Podbielski A, Horaud T, Ferrieri P. Identical genes confer high-level resistance to gentamicin upon *Enterococcus faecalis*, *Enterococcus faecium* and *Streptococcus agalactiae*. *Antimicrob Agents Chemother* 1992; **36:** 1215–1218.
99. Faibis F, Fiacre A, Demachy MC. Emergence of high-level gentamicin resistance in group G streptococci. *Eur J Clin Microbiol Infect Dis* 2001; **20:** 901–902.
100. Kaufhold A, Potgieter E. Chromosomally mediated high-level gentamicin resistance in *Streptococcus mitis*. *Antimicrob Agents Chemother* 1993; **37:** 2740–2742.
101. Kobayashi I, Kanayama A, Matsuzaki K, Nishida M, Nakatogawa N, Kaneko A. High-level gentamicin-resistant isolates of oral streptococci and *Aerococcus* from blood specimens. *J Infect Chemother* 2003; **9:** 21–24.
102. Kariyama R, Kumon H, Chow L *et al*. *In-vitro* activity of the combination of ampicillin and arbekacin against high-level gentamicin-resistant enterococci. *J Antimicrob Chemother* 1998; **42:** 836–838.
103. You I, Kariyama R, Zervos MJ, Kumon H, Chow JW. *In-vitro* activity of arbekacin alone and in combination with vancomycin against gentamicin- and methicillin-resistant *Staphylococcus aureus*. *Diagn Microbiol Infect Dis* 2000; **36:** 37–41.
104. Akins RL, Rybak MJ. *In vitro* activities of daptomycin, arbekacin, vancomycin, and gentamicin alone and/or in combination against glycopeptide intermediate-resistant *Staphylococcus aureus* in an infection model. *Antimicrob Agents Chemother* 2000; **44:** 1925–1929.
105. Kak V, Donabedian SM, Zervos MJ, Kariyama R, Kumon H, Chow JW. Efficacy of ampicillin plus arbekacin in experimental rabbit endocarditis caused by an *Enterococcus faecalis* strain with high-level gentamicin resistance. *Antimicrob Agents Chemother* 2000; **44:** 2545–2546.
106. Kao SJ, You I, Clewell DB *et al*. Detection of the high-level aminoglycoside resistance gene *aph*(2″)-*Ib* in *Enterococcus faecium*. *Antimicrob Agents Chemother* 2000; **44:** 2876–2879.
107. Chow JW, Kak V, You I *et al*. Aminoglycoside resistance genes *aph(2″)-Ib* and *aac(6′)-Im* detected together in strains of both *Escherichia coli* and *Enterococcus faecium*. *Antimicrob Agents Chemother* 2001; **45:** 2691–2694.
108. Chow JW, Zervos MJ, Lerner SA *et al*. A novel gentamicin resistance gene in *Enterococcus*. *Antimicrob Agents Chemother* 1997; **41:** 511–514.
109. Lee HK, Vakulenko SB, Clewell DB, Lerner SA, Chow JW. Mutations in the *aph*(2″)-*Ic* gene are responsible for increased levels of aminoglycoside resistance. *Antimicrob Agents Chemother* 2002; **46:** 3253–3256.
110. Tsai SF, Zervos MJ, Clewell DB, Donabedian SM, Sahm DF, Chow JW. A new high-level gentamicin resistance gene, *aph*(2″)-*Id*, in *Enterococcus* spp. *Antimicrob Agents Chemother* 1998; **42:** 1229–1232.
111. Kak V, You I, Zervos MJ, Kariyama R, Kumon H, Chow JW. *In-vitro* synergistic activity of the combination of ampicillin and arbekacin against vancomycin- and high-level gentamicin-resistant *Enterococcus faecium* with the *aph*(2″)-*Id* gene(1). *Diagn Microbiol Infect Dis* 2000; **37:** 297–299.
112. Ounissi H, Derlot E, Carlier C, Courvalin P. Gene homogeneity for aminoglycoside-modifying enzymes in gram-positive bacteria. *Antimicrob Agents Chemother* 1990; **34:** 2164–2168.
113. Leclercq R, Dutka-Malen S, Brisson-Noel A *et al*. Resistance of enterococci to aminoglycosides and glycopeptides. *Clin Infect Dis* 1992; **15:** 495–501.
114. Kobayashi N, Alam M, Nishimoto Y, Urasawa S, Uehara N, Watanabe N. Distribution of aminoglycoside resistance genes in recent clinical isolates of *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus avium*. *Epidemiol Infect* 2001; **126:** 197–204.
115. Carlier C, Courvalin P. Emergence of 4′,4″-aminoglycoside nucleotidyltransferase in enterococci. *Antimicrob Agents Chemother* 1990; **34:** 1565–1569.
116. Seral C, Castillo FJ, Rubio-Calvo MC, Fenoll A, Garcia C, Gomez-Lus R. Distribution of resistance genes *tet*(M), *aph3′-III*, *catpC194* and the integrase gene of Tn*1545* in clinical *Streptococcus pneumoniae* harbouring *erm*(B) and *mef*(A) genes in Spain. *J Antimicrob Chemother* 2001; **47:** 863–866.
117. Poyart-Salmeron C, Trieu-Cuot P, Carlier C, Courvalin P. Nucleotide sequences specific for Tn*1545*-like conjugative transposons in pneumococci and staphylococci resistant to tetracycline. *Antimicrob Agents Chemother* 1991; **35:** 1657–1660.
118. Caillaud F, Trieu-Cuot P, Carlier C, Courvalin P. Nucleotide sequence of the kanamycin resistance determinant of the pneumococcal transposon Tn*1545*: evolutionary relationships and transcriptional analysis of *aphA-3* genes. *Mol Gen Genet* 1987; **207:** 509–513.
119. Clark NC, Olsvik O, Swenson JM, Spiegel CA, Tenover FC. Detection of a streptomycin/spectinomycin adenylyltransferase gene (*aadA*) in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 1999; **43:** 157–160.
120. Farber BF, Yee Y. High-level aminoglycoside resistance mediated by aminoglycoside-modifying enzymes among viridans streptococci: implications for the therapy for endocarditis. *J Infect Dis* 1987; **155:** 948–953.
121. Galimand M, Lambert T, Gerbaud G, Courvalin P. High-level aminoglycoside resistance in the β-hemolytic group G *Streptococcus* isolate BM2721. *Antimicrob Agents Chemother* 1999; **43:** 3008–3010.
122. Werner G, Hildebrandt B, Witte W. Linkage of *erm*(B) and *aadE-sat4-aphA-3* in multiple-resistant *Enterococcus faecium* isolates of different ecological origins. *Microb Drug Resist* 2003; **9**(suppl 1)**:** 9–16.
123. Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B antibiotic resistance determinants. *Antimicrob Agents Chemother* 1999; **43:** 2823–2830.
124. Schlunzen F, Zarivach R, Harms J *et al*. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature* 2001; **413:** 814–821.
125. Liu M, Douthwaite S. Activity of the ketolide telithromycin is refractory to Erm monomethylation of bacterial rRNA. *Antimicrob Agents Chemother* 2002; **46:** 1629–1633.
126. Jalava J, Kataja J, Seppala H, Huovinen P. *In vitro* activities of the novel ketolide telithromycin (HMR 3647) against erythromycin-resistant *Streptococcus* species. *Antimicrob Agents Chemother* 2001; **45:** 789–793.
127. Ryan BM, Dougherty TJ, Beaulieu D, Chuang J, Dougherty BA, Barrett JF. Efflux in bacteria: what do we really

know about it? *Expert Opin Investig Drugs* 2001; **10:** 1409–1422.
128. Van Bambeke F, Glupczynski Y, Plesiat P, Pechere JC, Tulkens PM. Antibiotic efflux pumps in prokaryotic cells: occurrence, impact on resistance and strategies for the future of antimicrobial therapy. *J Antimicrob Chemother* 2003; **51:** 1055–1065.
129. Sutcliffe J, Tait-Kamradt A, Wondrack L. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. *Antimicrob Agents Chemother* 1996; **40:** 1817–1824.
130. Luna VA, Coates P, Eady EA, Cove JH, Nguyen TT, Roberts MC. A variety of gram-positive bacteria carry mobile *mef* genes. *J Antimicrob Chemother* 1999; **44:** 19–25.
131. Del Grosso M, Iannelli F, Messina C *et al.* Macrolide efflux genes *mef*(A) and *mef*(E) are carried by different genetic elements in *Streptococcus pneumoniae*. *J Clin Microbiol* 2002; **40:** 774–778.
132. Portillo A, Ruiz-Larrea F, Zarazaga M, Alonso A, Martinez JL, Torres C. Macrolide resistance genes in *Enterococcus* spp. *Antimicrob Agents Chemother* 2000; **44:** 967–971.
133. Werner G, Hildebrandt B, Witte W. The newly described *msrC* gene is not equally distributed among all isolates of *Enterococcus faecium*. *Antimicrob Agents Chemother* 2001; **45:** 3672–3673.
134. Tait-Kamradt A, Davies T, Cronan M, Jacobs MR, Appelbaum PC, Sutcliffe J. Mutations in 23S rRNA and ribosomal protein L4 account for resistance in pneumococcal strains selected *in vitro* by macrolide passage. *Antimicrob Agents Chemother* 2000; **44:** 2118–2125.
135. Nagai K, Appelbaum PC, Davies TA *et al.* Susceptibilities to telithromycin and six other agents and prevalence of macrolide resistance due to L4 ribosomal protein mutation among 992 pneumococci from 10 central and Eastern European countries. *Antimicrob Agents Chemother* 2002; **46:** 371–377.
136. Jones RN, Farrell DJ, Morrissey I. Quinupristin–dalfopristin resistance in *Streptococcus pneumoniae*: novel L22 ribosomal protein mutation in two clinical isolates from the SENTRY antimicrobial surveillance program. *Antimicrob Agents Chemother* 2003; **47:** 2696–2698.
137. Farrell DJ, Douthwaite S, Morrissey I *et al.* Macrolide resistance by ribosomal mutation in clinical isolates of *Streptococcus pneumoniae* from the PROTEKT 1999–2000 study. *Antimicrob Agents Chemother* 2003; **47:** 1777–1783.
138. Doktor SZ, Shortridge VD, Beyer JM, Flamm RK. Epidemiology of macrolide and/or lincosamide resistant *Streptococcus pneumoniae* clinical isolates with ribosomal mutations. *Diagn Microbiol Infect Dis* 2004; **49:** 47–52.
139. Vester B, Douthwaite S. Macrolide resistance conferred by base substitutions in 23S rRNA. *Antimicrob Agents Chemother* 2001; **45:** 1–12.
140. Pihlajamaki M, Kataja J, Seppala H *et al.* Ribosomal mutations in *Streptococcus pneumoniae* clinical isolates. *Antimicrob Agents Chemother* 2002; **46:** 654–658.
141. Haanpera M, Huovinen P, Jalava J. Detection and quantification of macrolide resistance mutations at positions 2058 and 2059 of the 23S rRNA gene by pyrosequencing. *Antimicrob Agents Chemother* 2005; **49:** 457–460.
142. Jalava J, Vaara M, Huovinen P. Mutation at the position 2058 of the 23S rRNA as a cause of macrolide resistance in *Streptococcus pyogenes*. *Ann Clin Microbiol Antimicrob* 2004; **3:** 5.
143. Malbruny B, Canu A, Bozdogan B *et al.* Resistance to quinupristin–dalfopristin due to mutation of L22 ribosomal protein in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002; **46:** 2200–2207.
144. Clancy J, Dib-Hajj F, Petitpas JW, Yuan W. Cloning and characterization of a novel macrolide efflux gene, *mreA*, from *Streptococcus agalactiae*. *Antimicrob Agents Chemother* 1997; **41:** 2719–2723.
145. Matsuoka M, Inoue M, Endo Y, Nakajima Y. Characteristic expression of three genes, *msr*(A), *mph*(C) and *erm*(Y), that confer resistance to macrolide antibiotics on *Staphylococcus aureus*. *FEMS Microbiol Lett* 2003; **220:** 287–293.
146. Allignet J, Loncle V, Mazodier P, El-Solh N. Nucleotide sequence of a staphylococcal plasmid gene, *vgb*, encoding a hydrolase inactivating the B components of virginiamycin-like antibiotics. *Plasmid* 1988; **20:** 271–275.
147. Allignet J, Liassine N, El-Solh N. Characterization of a staphylococcal plasmid related to pUB110 and carrying two novel genes, *vatC* and *vgbB*, encoding resistance to streptogramins A and B and similar antibiotics. *Antimicrob Agents Chemother* 1998; **42:** 1794–1798.
148. Mukhtar TA, Koteva KP, Hughes DW, Wright GD. *Vgb* from *Staphylococcus aureus* inactivates streptogramin B antibiotics by an elimination mechanism not hydrolysis. *Biochemistry* 2001; **40:** 8877–8886.
149. Jensen LB, Hammerum AM, Aarestrup FM, van den Bogaard AE, Stobberingh EE. Occurrence of *satA* and *vgb* genes in streptogramin-resistant *Enterococcus faecium* isolates of animal and human origins in The Netherlands. *Antimicrob Agents Chemother* 1998; **42:** 3330–3331.
150. Lina G, Quaglia A, Reverdy M-E, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides and streptogramins among staphylococci. *Antimicrob Agents Chemother* 1999; **43:** 1062–1066.
151. Loeza-Lara PD, Soto-Huipe M, Baizabal-Aguirre VM *et al.* pBMSa1, a plasmid from a dairy cow isolate of *Staphylococcus aureus*, encodes a lincomycin resistance determinant and replicates by the rolling-circle mechanism. *Plasmid* 2004; **52:** 48–56.
152. Bozdogan B, Berrezouga L, Kuo M-S *et al.* A new resistance gene, *linB*, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium*. *Antimicrob Agents Chemother* 1999; **43:** 925–929.
153. Farrell DJ, Jenkins SG. Distribution across the USA of macrolide resistance and macrolide resistance mechanisms among *Streptococcus pneumoniae* isolates collected from patients with respiratory tract infections: PROTEKT US 2001–2002. *J Antimicrob Chemother* 2004; **54**(suppl 1)**:** i17–i22.
154. Felmingham D, Shackcloth J, Tillotson G. BSAC Respiratory Resistance Surveillance Programme (2002–2003): comparative susceptibility of *Streptococcus pneumoniae*, cultured from patients in Great Britain and Ireland with community-acquired lower respiratory tract infection, to gemifloxacin. *J Antimicrob Chemother* 2004; **54:** 698–699.
155. El-Solh N, Allignet J. Staphylococcal resistance to streptogramins and related antibiotics. *Drug Resist Updat* 1998; **1:** 169–175.
156. Bonfiglio G, Furneri PM. Novel streptogramin antibiotics. *Expert Opin Investig Drugs* 2001; **10:** 185–198.

157. Singh KV, Weinstock GM, Murray BE. An *Enterococcus faecalis* ABC homologue (Lsa) is required for the resistance of this species to clindamycin and quinupristin–dalfopristin. *Antimicrob Agents Chemother* 2002; **46:** 1845–1850.
158. Dina J, Malbruny B, Leclercq R. Nonsense mutations in the *lsa*-like gene in *Enterococcus faecalis* isolates susceptible to lincosamides and streptogramins A. *Antimicrob Agents Chemother* 2003; **47:** 2307–2309.
159. Singh KV, Murray BE. Differences in the *Enterococcus faecalis lsa* locus that influence susceptibility to quinupristin–dalfopristin and clindamycin. *Antimicrob Agents Chemother* 2005; **49:** 32–39.
160. Johnson AP, Warner M, Hallas G, Livermore DM. Susceptibility to quinupristin/dalfopristin and other antibiotics of vancomycin-resistant enterococci from the UK, 1997 to mid-1999. *J Antimicrob Chemother* 2000; **46:** 125–128.
161. Bozdogan B, Leclercq R. Effects of genes encoding resistance to streptogramins A and B on the activity of quinupristin–dalfopristin against *Enterococcus faecium*. *Antimicrob Agents Chemother* 1999; **43:** 2720–2725.
162. Allignet J, Loncle V, Simenel C, Delepierre M, El-Solh N. Sequence of a staphylococcal gene, *vat*, encoding an acetyltransferase inactivating the A-type compounds of virginiamycin-like antibiotics. *Gene* 1993; **130:** 91–98.
163. Allignet J, El-Solh N. Diversity among the gram-positive acetyltransferases inactivating streptogramin A and structurally related compounds and characterization of a new staphylococcal determinant, *vatB*. *Antimicrob Agents Chemother* 1995; **39:** 2027–2036.
164. Rende-Fournier R, Leclercq R, Galimand M, Duval J, Courvalin P. Identification of the *satA* gene encoding a streptogramin A acetyltransferase in *Enterococcus faecium* BM4145. *Antimicrob Agents Chemother* 1993; **37:** 2119–2125.
165. Werner G, Witte W. Characterization of a new enterococcal gene, *satG*, encoding a putative acetyltransferase conferring resistance to streptogramin A compounds. *Antimicrob Agents Chemother* 1999; **43:** 1813–1814.
166. Allignet J, Loncle V, El-Solh N. Sequence of a staphylococcal plasmid gene, *vga*, encoding a putative ATP-binding protein involved in resistance to virginiamycin A-like antibiotics. *Gene* 1992; **117:** 45–51.
167. Allignet J, El-Solh N. Characterization of a new staphylococcal gene, *vgaB*, encoding a putative ABC transporter conferring resistance to streptogramin A and related compounds. *Gene* 1997; **202:** 133–138.
168. Allignet J, El-Solh N. Comparative analysis of staphylococcal plasmids carrying three streptogramin-resistance genes: *vat-vgb-vga*. *Plasmid* 1999; **42:** 134–138.
169. Liassine N, Allignet J, Morvan A, Aubert S, El-Solh N. Multiplicity of the genes and plasmids conferring resistance to pristinamycin in staphylococci in an Algerian hospital. *Zbl Bakt* 1997; **286:** 389–399.
170. Hammerum AM, Flannagan SE, Clewell DB, Jensen LB. Indication of transposition of a mobile DNA element containing the *vat*(D) and *erm*(B) genes in *Enterococcus faecium*. *Antimicrob Agents Chemother* 2001; **45:** 3223–3225.
171. Jensen LB, Hammerum AM, Aarestrup FM. Linkage of *vatE* and *ermB* in streptogramin resistant *Enterococcus faecium* isolates from Europe. *Antimicrob Agents Chemother* 2000; **44:** 2231–2232.
172. Bozdogan B, Leclercq R, Lozniewski A, Weber M. Plasmid-mediated coresistance to streptogramins and vancomycin in *Enterococcus faecium* HM1032. *Antimicrob Agents Chemother* 1999; **43:** 2097–2098.
173. Werner G, Klare I, Spencker FB, Witte W. Intra-hospital dissemination of quinupristin/dalfopristin- and vancomycin-resistant *Enterococcus faecium* in a paediatric ward of a German hospital. *J Antimicrob Chemother* 2003; **52:** 113–115.
174. Werner G, Klare I, Witte W. Association between quinupristin/dalfopristin resistance in glycopeptide-resistant *Enterococcus faecium* and the use of additives in animal feed. *Eur J Clin Microbiol Infect Dis* 1998; **17:** 401–402.
175. Werner G, Klare I, Heier H *et al*. Quinupristin/dalfopristin-resistant enterococci of the satA (vatD) and satG (vatE) genotypes from different ecological origins in Germany. *Microb Drug Resist* 2000; **6:** 37–47.
176. Welton LA, Thal LA, Perri MB *et al*. Antimicrobial resistance in enterococci isolated from Turkey flocks fed virginiamycin. *Antimicrob Agents Chemother* 1998; **42:** 705–708.
177. Thal LA, Zervos MJ. Occurrence and epidemiology of resistance to virginiamycin and streptogramins. *J Antimicrob Chemother* 1999; **43:** 171–176.
178. Hammerum AM, Jensen LB, Aarestrup FM. Detection of the *satA* gene and transferability of virginiamycin resistance in *Enterococcus faecium* from food animals. *FEMS Microbiol Lett* 1998; **168:** 145–151.
179. Simjee S, McDermott PF, Wagner DD, White DG. Variation within the *vat*(E) allele of *Enterococcus faecium* isolates from retail poultry samples. *Antimicrob Agents Chemother* 2001; **45:** 2931–2932.
180. Simjee S, White DG, Wagner DD *et al*. Identification of *vat*(E) in *Enterococcus faecalis* isolates from retail poultry and its transferability to *Enterococcus faecium*. *Antimicrob Agents Chemother* 2002; **46:** 3823–3828.
181. Soltani M, Beighton D, Philpott-Howard J, Woodford N. Mechanisms of resistance to quinupristin/dalfopristin among isolates of *Enterococcus faecium* from animals, raw meat and hospital patients in Western Europe. *Antimicrob Agents Chemother* 2000; **44:** 433–436.
182. Soltani M, Beighton D, Philpott-Howard J, Woodford N. Identification of *vat*(E-3), a novel gene encoding resistance to quinupristin-dalfopristin in a strain of *Enterococcus faecium* from a hospital patient in the United Kingdom. *Antimicrob Agents Chemother* 2001; **45:** 645–646.
183. Appelbaum PC, Hunter PA. The fluoroquinolone antibacterials: past, present and future perspectives. *Int J Antimicrob Agents* 2000; **16:** 5–15.
184. Hooper DC. Emerging mechanisms of fluoroquinolone resistance. *Emerg Infect Dis* 2001; **7:** 337–341.
185. Hooper DC. Fluoroquinolone resistance among Gram-positive cocci. *Lancet Infect Dis* 2002; **2:** 530–538.
186. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother* 2003; **51:** 1109–1117.
187. Korten V, Huang WM, Murray BE. Analysis by PCR and direct DNA sequencing of *gyrA* mutations associated with fluoroquinolone resistance in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 1994; **38:** 2091–2094.
188. Kanematsu E, Deguchi T, Yasuda M, Kawamura T, Nishino Y, Kawada Y. Alterations in the GyrA subunit of DNA gyrase and the ParC subunit of DNA topoisomerase IV associated with quinolone resistance in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 1998; **42:** 433–435.

189. Brisse S, Fluit AC, Wagner U *et al.* Association of alterations in ParC and GyrA proteins with resistance of clinical isolates of *Enterococcus faecium* to nine different fluoroquinolones. *Antimicrob Agents Chemother* 1999; **43:** 2513–2516.
190. el Amin NA, Jalal S, Wretlind B. Alterations in GyrA and ParC associated with fluoroquinolone resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* 1999; **43:** 947–949.
191. Onodera Y, Okuda J, Tanaka M, Sato K. Inhibitory activities of quinolones against DNA gyrase and topoisomerase IV of *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2002; **46:** 1800–1804.
192. Petersen A, Jensen LB. Analysis of *gyrA* and *parC* mutations in enterococci from environmental samples with reduced susceptibility to ciprofloxacin. *FEMS Microbiol Lett* 2004; **231:** 73–76.
193. Yan SS, Fox ML, Holland SM, Stock F, Gill VJ, Fedorko DP. Resistance to multiple fluoroquinolones in a clinical isolate of *Streptococcus pyogenes*: identification of *gyrA* and *parC* and specification of point mutations associated with resistance. *Antimicrob Agents Chemother* 2000; **44:** 3196–3198.
194. Richter SS, Diekema DJ, Heilmann KP *et al.* Fluoroquinolone resistance in *Streptococcus pyogenes*. *Clin Infect Dis* 2003; **36:** 380–383.
195. Reinert RR, Lutticken R, Al Lahham A. High-level fluoroquinolone resistance in a clinical *Streptococcus pyogenes* isolate in Germany. *Clin Microbiol Infect* 2004; **10:** 659–662.
196. Yoshida H, Bogaki M, Nakamura S, Ubukata K, Konno M. Nucleotide sequence and characterization of the *Staphylococcus aureus norA* gene, which confers resistance to quinolones. *J Bacteriol* 1990; **172:** 6942–6949.
197. Neyfakh AA, Borsch CM, Kaatz GW. Fluoroquinolone resistance protein NorA of *Staphylococcus aureus* is a multidrug efflux transporter. *Antimicrob Agents Chemother* 1993; **37:** 128–129.
198. Truong-Bolduc QC, Zhang X, Hooper DC. Characterization of NorR protein, a multifunctional regulator of *norA* expression in *Staphylococcus aureus*. *J Bacteriol* 2003; **185:** 3127–3138.
199. Gibbons S, Oluwatuyi M, Kaatz GW. A novel inhibitor of multidrug efflux pumps in *Staphylococcus aureus*. *J Antimicrob Chemother* 2003; **51:** 13–17.
200. Kaatz GW, Moudgal VV, Seo SM. Identification and characterization of a novel efflux-related multidrug resistance phenotype in *Staphylococcus aureus*. *J Antimicrob Chemother* 2002; **50:** 833–838.
201. Noguchi N, Okada H, Narui K, Sasatsu M. Comparison of the nucleotide sequence and expression of norA genes and microbial susceptibility in 21 strains of *Staphylococcus aureus*. *Microb Drug Resist* 2004; **10:** 197–203.
202. Gill MJ, Brenwald NP, Wise R. Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 1999; **43:** 187–189.
203. Brenwald NP, Appelbaum P, Davies T, Gill MJ. Evidence for efflux pumps, other than PmrA, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. *Clin Microbiol Infect* 2003; **9:** 140–143.
204. Brenwald NP, Gill MJ, Wise R. The effect of reserpine, an inhibitor of multi-drug efflux pumps, on the *in-vitro* susceptibilities of fluoroquinolone-resistant strains of *Streptococcus pneumoniae* to norfloxacin. *J Antimicrob Chemother* 1997; **40:** 458–459.
205. Piddock LJ, Johnson MM. Accumulation of 10 fluoroquinolones by wild-type or efflux mutant *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2002; **46:** 813–820.
206. Pestova E, Millichap JJ, Siddiqui F, Noskin GA, Peterson LR. Non-PmrA-mediated multidrug resistance in *Streptococcus pneumoniae*. *J Antimicrob Chemother* 2002; **49:** 553–556.
207. Piddock LJ, Johnson MM, Simjee S, Pumbwe L. Expression of efflux pump gene *pmrA* in fluoroquinolone-resistant and -susceptible clinical isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2002; **46:** 808–812.
208. Davis DR, McAlpine JB, Pazoles CJ *et al.* *Enterococcus faecalis* multi-drug resistance transporters: application for antibiotic discovery. *J Mol Microbiol Biotechnol* 2001; **3:** 179–184.
209. Jonas BM, Murray BE, Weinstock GM. Characterization of *emeA*, a *norA* homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2001; **45:** 3574–3579.
210. Ince D, Hooper DC. Quinolone resistance due to reduced target enzyme expression. *J Bacteriol* 2003; **185:** 6883–6892.
211. Ford CW, Zurenko GE, Barbachyn MR. The discovery of linezolid, the first oxazolidinone antibacterial agent. *Curr Drug Targets Infect Disord* 2001; **1:** 181–199.
212. Zhou CC, Swaney SM, Shinabarger DL, Stockman BJ. ^{1}H Nuclear magnetic resonance study of oxazolidinone binding to bacterial ribosomes. *Antimicrob Agents Chemother* 2002; **46:** 625–629.
213. Colca JR, McDonald WG, Waldon DJ *et al.* Crosslinking in the living cell locates the site of action of oxazolidinone antibiotics. *J Biol Chem* 2003; **278:** 21972–21979.
214. Prystowsky J, Siddiqui F, Chosay J *et al.* Resistance to linezolid: characterization of mutations in rRNA and comparison of their occurrences in vancomycin-resistant enterococci. *Antimicrob Agents Chemother* 2001; **45:** 2154–2156.
215. Auckland C, Teare L, Cooke F *et al.* Linezolid-resistant enterococci: report of the first isolates in the United Kingdom. *J Antimicrob Chemother* 2002; **50:** 743–746.
216. Johnson AP, Tysall L, Stockdale MW *et al.* Emerging linezolid resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from two Austrian patients in the same intensive care unit. *Eur J Clin Microbiol Infect Dis* 2002; **21:** 751–754.
217. Halle E, Padberg J, Rosseau S, Klare I, Werner G, Witte W. Linezolid-resistant *Enterococcus faecium* and *Enterococcus faecalis* isolated from a septic patient: report of first isolates in Germany. *Infection* 2004; **32:** 182–183.
218. Bersos Z, Maniati M, Kontos F, Petinaki E, Maniatis AN. First report of a linezolid-resistant vancomycin-resistant *Enterococcus faecium* strain in Greece. *J Antimicrob Chemother* 2004; **53:** 685–686.
219. Tsiodras S, Gold HS, Sakoulas G *et al.* Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. *Lancet* 2001; **358:** 207–208.
220. Wilson P, Andrews JA, Charlesworth R *et al.* Linezolid resistance in clinical isolates of *Staphylococcus aureus*. *J Antimicrob Chemother* 2003; **51:** 186–188.
221. Machado ARL, Andrade SS, Barth AL, Lutz L, Sader HS, Gales AC. The emergence of linezolid-resistance among

Staphylococcus aureus from cystic fibrosis patients [abstract C2–1825]. In: *Abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Chicago, IL: American Society for Microbiology, 2003; 144.
222. Enne V, Howe RA, Walsh TR, Mutnick AH, Jones RN. Initial descriptions of linezolid resistance in *Staphylococcus epidermidis* and *Streptococcus oralis*: report from the SENTRY antimicrobial surveillance program [abstract LB-10]. In: *Abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy*. San Diego, CA: American Society for Microbiology, 2002.
223. Meka VG, Pillai SK, Sakoulas G *et al*. Linezolid resistance in sequential *Staphylococcus aureus* isolates associated with a T2500A mutation in the 23S rRNA gene and loss of a single copy of rRNA. *J Infect Dis* 2004; **190:** 311–317.
224. Marshall SH, Donskey CJ, Hutton-Thomas R, Salata RA, Rice LB. Gene dosage and linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2002; **46:** 3334–3336.
225. Sinclair A, Arnold C, Woodford N. Rapid detection and estimation by pyrosequencing of 23S rRNA genes with a single nucleotide polymorphism conferring linezolid resistance in enterococci. *Antimicrob Agents Chemother* 2003; **47:** 3620–3622.
226. Ruggero KA, Schroeder LK, Schreckenberger PC, Mankin AS, Quinn JP. Nosocomial superinfections due to linezolid-resistant *Enterococcus faecalis*: evidence for a gene dosage effect on linezolid MICs. *Diagn Microbiol Infect Dis* 2003; **47:** 511–513.
227. Lobritz M, Hutton-Thomas R, Marshall S, Rice LB. Recombination proficiency influences frequency and locus of mutational resistance to linezolid in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2003; **47:** 3318–3320.
228. Jones RN, Della-Latta P, Lee LV, Beidenbach DJ. Linezolid-resistant *Enterococcus faecium* isolated from a patient without prior exposure to an oxazolidinone: report from the SENTRY Antimicrobial Surveillance Program. *Diagn Microbiol Infect Dis* 2002; **42:** 137–139.
229. Gonzales RD, Schreckenberger PC, Graham MB, Kelkar S, DenBesten K, Quinn JP. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. *Lancet* 2001; **357:** 1179.
230. Engemann JJ, Joyce MJ, Harrell LJ *et al*. Outbreak of linezolid-resistant *Enterococcus faecium* bloodstream infections on an oncology ward [abstract K-1112]. In: *Abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Chicago, IL: American Society for Microbiology, 2003; 359.
231. Trieu-Cuot P, de Cespedes G, Bentorcha F, Delbos F, Gaspar E, Horaud T. Study of heterogeneity of chloramphenicol acetyltransferase (CAT) genes in streptococci and enterococci by polymerase chain reaction: characterization of a new CAT determinant. *Antimicrob Agents Chemother* 1993; **37:** 2593–2598.
232. Pozzi G, Guild WR. Two genes for chloramphenicol resistance common to staphylococci and streptococci. *Eur J Epidemiol* 1988; **4:** 20–24.
233. Widdowson CA, Klugman KP. Molecular mechanisms of resistance to commonly used non-β-lactam drugs in *Streptococcus pneumoniae*. *Semin Respir Infect* 1999; **14:** 255–268.
234. Widdowson CA, Adrian PV, Klugman KP. Acquisition of chloramphenicol resistance by the linearization and integration of the entire staphylococcal plasmid pC194 into the chromosome of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2000; **44:** 393–395.
235. Pepper K, de Cespedes G, Horaud T. Heterogeneity of chromosomal genes encoding chloramphenicol resistance in streptococci. *Plasmid* 1988; **19:** 71–74.
236. Brown EM, Thomas P. Fusidic acid resistance in *Staphylococcus aureus* isolates. *Lancet* 2002; **359:** 803.
237. Nagaev I, Bjorkman J, Andersson DI, Hughes D. Biological cost and compensatory evolution in fusidic acid-resistant *Staphylococcus aureus*. *Mol Microbiol* 2001; **40:** 433–439.
238. Besier S, Ludwig A, Brade V, Wichelhaus TA. Biological cost of fusidic acid resistance in *Staphylococcus aureus* [abstract P1721]. In: *Abstracts of the 14th European Congress of Clinical Microbiology and Infectious Diseases*. Prague, Czech Republic: European Society of Clinical Microbiology and Infectious Diseases, 2004; 488.
239. Turnidge J, Collignon P. Resistance to fusidic acid. *Int J Antimicrob Agents* 1999; **12**(suppl 2)**:** S35–S44.
240. Besier S, Ludwig A, Brade V, Wichelhaus TA. Molecular analysis of fusidic acid resistance in *Staphylococcus aureus*. *Mol Microbiol* 2003; **47:** 463–469.
241. Mason BW, Howard AJ, Magee JT. Fusidic acid resistance in community isolates of methicillin-susceptible *Staphylococcus aureus* and fusidic acid prescribing. *J Antimicrob Chemother* 2003; **51:** 1033–1036.
242. O'Neill AJ, Larsen AR, Henriksen AS, Chopra I. A fusidic acid-resistant epidemic strain of *Staphylococcus aureus* carries the *fusB* determinant, whereas *fusA* mutations are prevalent in other resistant isolates. *Antimicrob Agents Chemother* 2004; **48:** 3594–3597.
243. O'Brien FG, Price C, Grubb WB, Gustafson JE. Genetic characterization of the fusidic acid and cadmium resistance determinants of *Staphylococcus aureus* plasmid pUB101. *J Antimicrob Chemother* 2002; **50:** 313–321.
244. Gilbart J, Perry CR, Slocombe B. High-level mupirocin resistance in *Staphylococcus aureus*: evidence for two distinct isoleucyl-tRNA synthetases. *Antimicrob Agents Chemother* 1993; **37:** 32–38.
245. Antonio M, McFerran N, Pallen MJ. Mutations affecting the Rossman fold of isoleucyl-tRNA synthetase are correlated with low-level mupirocin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002; **46:** 438–442.
246. Dyke KGH, Curnock SP, Golding M, Noble WC. Cloning of the gene conferring resistance to mupirocin in *Staphylococcus aureus*. *FEMS Microbiol Lett* 1991; **77:** 195–198.
247. Hodgson JE, Curnock SP, Dyke KGH, Morris R, Sylvester DR, Gross MS. Molecular characterization of the gene encoding high-level mupirocin resistance in *Staphylococcus aureus* J2870. *Antimicrob Agents Chemother* 1994; **38:** 1205–1208.
248. Woodford N, Watson AP, Patel S, Jevon M, Waghorn DJ, Cookson BD. Heterogeneous location of the *mupA* high-level mupirocin resistance gene in *Staphylococcus aureus*. *J Med Microbiol* 1998; **47:** 829–835.
249. Aubry-Damon H, Soussy CJ, Courvalin P. Characterization of mutations in the *rpoB* gene that confer rifampin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1998; **42:** 2590–2594.
250. Wichelhaus TA, Schafer V, Brade V, Boddinghaus B. Molecular characterization of *rpoB* mutations conferring

cross-resistance to rifamycins on methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1999; **43:** 2813–2816.
251. Wichelhaus T, Schafer V, Brade V, Boddinghaus B. Differential effect of *rpoB* mutations on antibacterial activities of rifampicin and KRM-1648 against *Staphylococcus aureus*. *J Antimicrob Chemother* 2001; **47:** 153–156.
252. Enne VI, Delsol AA, Roe JM, Bennett PM. Rifampicin resistance and its fitness cost in *Enterococcus faecium*. *J Antimicrob Chemother* 2004; **53:** 203–207.
253. Aubry-Damon H, Galimand M, Gerbaud G, Courvalin P. *rpoB* mutation conferring rifampin resistance in *Streptococcus pyogenes*. *Antimicrob Agents Chemother* 2002; **46:** 1571–1573.
254. Tanaka Y, Yazawa K, Dabbs ER *et al*. Different rifampicin inactivation mechanisms in *Nocardia* and related taxa. *Microbiol Immunol* 1996; **40:** 1–4.
255. Houang ET, Chu YW, Lo WS, Chu KY, Cheng AF. Epidemiology of rifampin ADP-ribosyltransferase (*arr-2*) and metallo-β-lactamase (bla_{IMP-4}) gene cassettes in class 1 integrons in *Acinetobacter* strains isolated from blood cultures in 1997 to 2000. *Antimicrob Agents Chemother* 2003; **47:** 1382–1390.
256. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* 2001; **65:** 232–260.
257. Connell SR, Tracz DM, Nierhaus KH, Taylor DE. Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob Agents Chemother* 2003; **47:** 3675–3681.
258. Trzcinski K, Cooper BS, Hryniewicz W, Dowson CG. Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2000; **45:** 763–770.
259. Huys G, D'Haene K, Collard JM, Swings J. Prevalence and molecular characterization of tetracycline resistance in *Enterococcus* isolates from food. *Appl Environ Microbiol* 2004; **70:** 1555–1562.
260. Tuckman M, Petersen PJ, Projan SJ. Mutations in the interdomain loop region of the *tetA*(A) tetracycline resistance gene increase efflux of minocycline and glycylcyclines. *Microb Drug Resist* 2000; **6:** 277–282.
261. Guay GG, Tuckman M, Rothstein DM. Mutations in the *tetA*(B) gene that cause a change in substrate specificity of the tetracycline efflux pump. *Antimicrob Agents Chemother* 1994; **38:** 857–860.
262. Visalli MA, Murphy E, Projan SJ, Bradford PA. AcrAB multidrug efflux pump is associated with reduced levels of susceptibility to tigecycline (GAR-936) in *Proteus mirabilis*. *Antimicrob Agents Chemother* 2003; **47:** 665–669.
263. Adrian PV, Klugman KP. Mutations in the dihydrofolate reductase gene of trimethoprim-resistant isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 1997; **41:** 2406–2413.
264. Dale GE, Broger C, D'Arcy A *et al*. A single amino acid substitution in *Staphylococcus aureus* dihydrofolate reductase determines trimethoprim resistance. *J Mol Biol* 1997; **266:** 23–30.
265. Coque TM, Singh KV, Weinstock GM, Murray BE. Characterization of dihydrofolate reductase genes from trimethoprim-susceptible and trimethoprim-resistant strains of *Enterococcus faecalis*. *Antimicrob Agents Chemother* 1999; **43:** 141–147.
266. Dale GE, Broger C, Hartman PG *et al*. Characterization of the gene for the chromosomal dihydrofolate reductase (DHFR) of *Staphylococcus epidermidis* ATCC 14990: the origin of the trimethoprim-resistant S1 DHFR from *Staphylococcus aureus*? *J Bacteriol* 1995; **177:** 2965–2970.
267. Dale GE, Langen H, Page MG, Then RL, Stuber D. Cloning and characterization of a novel, plasmid-encoded trimethoprim-resistant dihydrofolate reductase from *Staphylococcus haemolyticus* MUR313. *Antimicrob Agents Chemother* 1995; **39:** 1920–1924.
268. Woodford N, Morrison D, Cookson B, George RC. Comparison of high-level gentamicin-resistant *Enterococcus faecium* isolates from different continents. *Antimicrob Agents Chemother* 1993; **37:** 681–684.
269. Frosolono M, Hodel-Christian SL, Murray BE. Lack of homology of enterococci which have high-level resistance to trimethoprim with the *dfrA* gene of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1991; **35:** 1928–1930.